DOI:
10.1177/1178646917691739

Document Version
Publisher's PDF, also known as Version of record

Nicotinamide *N*-Methyltransferase in Health and Cancer

David B Ramsden[1], Rosemary H Waring[2], David J Barlow[3] and Richard B Parsons[3]

[1]Institute of Metabolism and Systems Research, The Medical School, University of Birmingham, Birmingham, UK. [2]School of Biosciences, University of Birmingham, Birmingham, UK. [3]Institute of Pharmaceutical Science, King's College London, London, UK.

International Journal of Tryptophan Research
Volume 10: 1–19

DOI: 10.1177/1178646917691739
SAGE

ABSTRACT: Over the past decade, the roles of nicotinamide *N*-methyltransferase and its product 1-methyl nicotinamide have emerged from playing merely minor roles in phase 2 xenobiotic metabolism as actors in some of the most important scenes of human life. In this review, the structures of the gene, messenger RNA, and protein are discussed, together with the role of the enzyme in many of the common cancers that afflict people today.

KEYWORDS: Energy metabolism, liver, fat, cancer

RECEIVED: November 28, 2016. **ACCEPTED:** January 11, 2017.

TYPE: Review

PEER REVIEW: Two peer reviewers contributed to the peer review report. Reviewers' reports totalled 1199 words, excluding any confidential comments to the academic editor.

FUNDING: The authors received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: David B Ramsden, Institute of Metabolism and Systems Research, The Medical School, University of Birmingham, Birmingham B15 2TT, UK. Email: d.b.ramsden@bham.ac.uk

Introduction

Methytransferases were some of the first enzymes to evolve, as can be seen from a brief inspection of the entries for methyltransferases in archaea in PubMed (Protein Clusters). Furthermore, a search for nicotinamide *N*-methyltransferase (NNMT) returned 106 results for NNMT-related proteins (as of the date of writing) in EnsemblGenomes showing the presence of these enzymes in a wide variety of species, which includes both animals and plants.[1] The *N*-methyltransferases comprise a group of some 30 or more enzymes which includes enzymes methylating amino groups of amino acids and amino acid residues in proteins, among which the latter class is histone methyltransferase, an enzyme involved in gene regulation. The evolution of oxidised nicotinamide adenine dinucleotide (NAD^+) synthesis and salvage,[2–4] the phylogenic distribution of NAD^+,[5] and the linkage between NAD^+ and adenosine triphosphate (ATP) synthesis have been explored as sequence data across many species have become available. These data have led to the conclusion that the emergence of new NAD^+-consuming enzymes increased and that NNMT probably emerged as a means of regulating levels of nicotinamide (NAM) within the cell, possibly so that NAM did not inhibit DNA repair.[6,7]

The Early History of NNMT

Methylation of a pyridine by a factor in human tissue was first described by His[8] in 1884. Nevertheless, for most of the first half of the 20th century, the metabolic fate of NAM was unclear, with trigonelline often being proposed as its principal final outcome. This was because the means of distinguishing 1-methylnicotinamide (MeNAM) from other compounds were not available. The conversion of NAM to MeNAM in human and animal tissues was shown to occur by the early 1940s. Ellinger and Coulson[9] described the excretion of MeNAM in human urine. That MeNAM is the primary urinary excretion product of human NAM metabolism was shown by Ellinger and Abdel Kader.[10] Further catabolism of MeNAM to pyridones was reported by Knox and Grossman[11] and Holman and Wiegand.[12] As early as 1941, Mann and Quastel[13] were exploring the interaction of NAM with *S*-adenosyl methionine (SAM). Again, in the 1940s, the major site of conversion of NAM to MeNAM was the liver.[14] The first detailed characterisation of a soluble protein enzyme capable of converting NAM to MeNAM was conducted by Cantoni[15] in 1951 using extracts from rat and pig livers.

NNMT: Gene, Messenger RNA, and Protein (NNMT: EC 2.1.1.1)

The human *NNMT* gene is contained on a 55.5-kb stretch on the forward strand of the long arm of chromosome 11 (11q23.1).[16] The gene contains either 3 or 5 exons depending on the transcription start site (TSS) used to transcribe messenger RNA (mRNA).[16–18] Of the 2 TSSs, the TSS further from the translation initiation ATG codon (TSS1) gives rise to a 1867 base transcript involving 5 exons, whereas the 3′ TSS (TSS2) gives rise to a 1610 base transcript involving 3 exons. Both transcripts give rise to the same translated sequence of 792 bases plus a TGA stop codon. Several other noncoding transcripts are also produced.[18] The 5′ flanking region of the gene from TSS2 contains numerous response elements, revealed by the TFSearch programme.[19] These include sites such as CdxA, GATA-1, CREB, CRE-BP, and AP-1 sites, although there is no TAATA site near the TSS. *Trans*-acting factors known to influence level of expression via transcription include hepatocyte nuclear factor-1β,[20] signal transducer and activator of transcription 3 (STAT3),[21] transforming growth factor β_1 (TGF-β_1),[22] and androgens.[23]

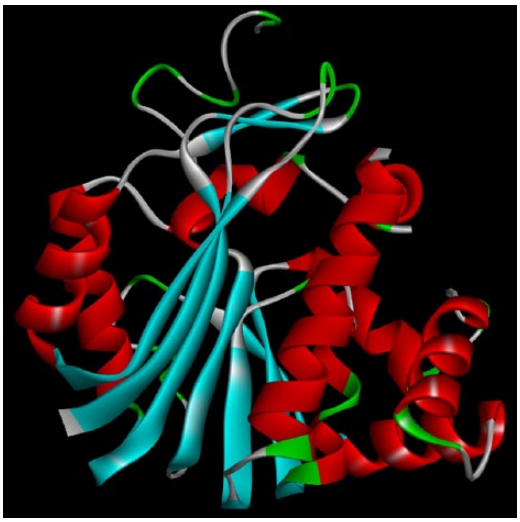

Figure 1. Ribbon cartoon of 3-dimensional structure of nicotinamide *N*-methyltransferase. Red denotes α-helix; blue, β-strand; grey, coil; and green, turn.

Endogenous c-Jun, in addition to any effect on mRNA levels, influences exon splicing of the pre-mRNA in mammary cancer.[24]

DNA analyses of samples taken from human populations across the world have revealed several hundred single-nucleotide polymorphisms across the gene and its flanking regions.[18] Nevertheless, no polymorphism in the exons of the 3-exon gene itself or in the immediate 5′ flanking region of TSS1 was found to be related to the level of protein expression.[25] Even so, numerous genetic polymorphisms not affecting the mRNA and protein structures have been reported to be linked to ageing[26]; cancer, eg, acute lymphoblastic leukaemia in children (intervening sequence – 151)[27,28] and oral squamous cell carcinoma[29]; and other diseases, eg, congenital heart defects in children (rs694539)[30] and bipolar disorder (rs694539).[31]

The NNMT protein comprises a single 264-amino-acid polypeptide of molecular weight 29 574.02 g/mol. It was isolated first from human liver homogenates by Aksoy et al.[17] Where the molecular weight has been investigated using sodium dodecyl sulphate polyacrylamide gel electrophoresis/western blotting, most reports do not state any difference from the figure of approximately 26000. Nevertheless, Lim et al[32] describe a number of posttranslational modifications to the protein in gastric cancer tissue. The primary sequence of the enzyme is closely related to that of 2 other *N*-methyltransferases, indolethylamine-*N*-methyltransferase and phenylethanolamine *N*-methyltransferase. These 3 enzymes form a subgroup within the large *N*-methyltransferase family.

The tissue with the highest level of expression in humans is the liver, as noted above.[17] Other sites of high expression include the kidney and lung[17] and white adipose tissue.[33] Expression of NNMT within the brain is largely confined to neurons and is highly regional. Sites of expression with the brain include the dopaminergic neurons of the substantia nigra and neurons in the granular layer of the cerebellum[34] and in the frontal cortex.[35] Expression within astrocytes is detectable but at very low levels.[36] Expression within the liver of normal individuals was said to have a bimodal distribution, with 25% of the population being high expressors and the remaining 75% being spread between moderate and low levels of expression, with an approximately 5-fold difference between the highest and lowest expressors.[37] In a study of mRNA sequences and protein levels isolated from sections of livers taken from 20 healthy individuals dying as a result of road traffic accidents or similar traumatic events, which were being used for liver transplantation, it was found that irrespective of the level of protein expression, all mRNAs had the same sequence. Furthermore, the protein expression followed a pattern of 1:2:1, high:intermediate:low ratio, in Hardy-Weinberg equilibrium.[38]

Nicotinamide *N*-methyltransferase uses SAM as the methyl donor to methylate the target amino group of the substrate and yield *S*-adenosyl homocysteine (SAH) which is converted to homocysteine and adenosine.[39] As might be expected, the 3-dimensional (3D) structure of NNMT contains the characteristic *N*-methyltransferase fold, where the substrate and the SAM cofactor are embedded deep within the molecule. The entrance to these sites is partially covered by a cap. This cap is formed by 2 α-helices near the *N*-terminus and a reverse β-hairpin spanning lysine residue 203 to serine residue 212.[40] The binding sites themselves are formed from 7 β-chains and 5 α-helices, the SAM-binding site having 3 β-strands and 2 α-helices, and the substrate-binding site having 4 β-strands and 2 α-helices with the 2 binding sites being separated by 1 α-helix.[40] In a crystallographic study by Peng et al, using NAM and SAH – the immediate product following donation of the methyl group of SAM – as ligands for the substrate and cofactor-binding sites, respectively, the residues involved in binding of the substrate and cofactor were identified.[41] The cofactor is in contact with more residues than is the substrate, which would tend to suggest that the cofactor is bound more strongly than the substrate. Tyrosine residues constitute a significant proportion of each of the binding sites. Figure 1 shows a ribbon cartoon of the human NNMT. Donation of the methyl group of SAM to NAM involves rearrangement of a major part of the protein's 3D structure (residues 134-154). This is to bring the donor and substrate in close proximity to allow the transfer of the methyl group to occur.[41]

NNMT and MeNAM in Biological Fluids

Although NNMT is an intracellular enzyme, it is released into the bloodstream, presumably chiefly from the liver. The median serum NNMT concentration of 25 healthy donors was reported to be 165 ng/L.[42] Other estimates of the serum concentration of MeNAM and urinary excretion of healthy humans are variously quoted as having a mean concentration of 96 ± 28 μmol/L (mean ± SD, N = 16) and a urinary excretion rate of 20 ± 5

μmol/24 hours (mean ± SD, N = 16)[43] or a mean serum concentration of 150 μmol/L and a mean 24-hour excretion rate of 48.8 μmol/24 hours.[44] As stated above, the liver is the major site of expression. Whether the genetically determined level of NNMT expression in the liver influences the levels of MeNAM in readily available fluids was uncertain. Therefore, one of us (R.H.W.) conducted a study of NAM metabolite excretion of 30 individuals in 8 family groups. The only metabolite of NAM that exhibited an excretion pattern resembling the expression pattern of high, intermediate, and low expression of NNMT in liver was MeNAM.[45] The study was conducted over a 24-hour period, thus obviating any effect that the circadian rhythm of NAD^+ synthesis might have on the results.[46] Nicotinamide *N*-methyltransferase expression itself is not thought to have a circadian rhythm. Whether all tissues in the body follow the pattern of NNMT expression seen in the liver is unclear, but in healthy individuals leading a normal life, the pattern of MeNAM excretion does appear to follow that of hepatic NNMT expression. Nevertheless, the lack of biopsy material meant that the 2 parameters could not be correlated.

1-Methylnicotinamide is actively taken into and released from cells. The uptake process in rat liver was first described by Moseley et al,[47] who characterised MeNAM/H^+ exchange in basolateral vesicles. Subsequently, the proteins that perform these processes have been recognised as members of a large class of ubiquitously expressed organic anion transporters (OATs), in particular OATs 1-3, which are capable of transporting a broad spectrum of ions.[48,49]

Ontogeny of NNMT

The ontogeny of the various enzymes of the phase 2 xenobiotic excretion system in mice has been elucidated in a study by Lu et al.[50] They showed that NNMT mRNA in the liver was present in only very low levels in prenatal animals. After birth, expression remained low until day 10, and thereafter, expression of the enzyme increased rapidly. By postnatal day 60, NNMT mRNA expression had reached its maximum. It would seem likely that a similar pattern of limited prenatal expression followed by rapidly increasing postnatal levels occurs in humans. Nevertheless, although in the neonatal animal NNMT appears to have limited expression, in the very earliest stage of human life, the enzyme does play a vital role. In human-naive embryonic stem cells, NNMT and MeNAM regulate pluripotency where the enzyme consumes SAM, thus limiting histone methylation. Hence, the enzyme and its product play an important role in regulating 'the epigenetic landscape of the earliest steps in human development'.[51]

NNMT and Ageing

In a review of the interplay of genetics and epigenetic signals in the developing neocortex, Huffmann[52] suggests that although genetics may be the dominant influence in early life, the influence of epigenetics may be significantly greater in the ageing process. This comes from work showing that caloric restriction influences DNA methylation, and hence ageing,[53,54] and earlier work linking caloric restriction to NAM, NAD^+, and sirtuins.[55–57] The interplay of these factors has been reviewed extensively by Imai and Guarente.[58] Importantly, from the point of view of this review, Schmeisser et al show that the methylation of NAM, as well as that of DNA, plays a significant role. They found that a low dose (1 μM) of MeNAM increased the life span of *Caenorhabditis elegans*, whereas a high dose (1 mM) had the opposite effect. In particular, they showed that the action of NAM was dependent on the conversion to MeNAM by this species' equivalent of mammalian NNMT, not by NAM modulating histone deacetylation by the nematodal orthologue of sirtuin 1. Cloning human NNMT into the nematode also increased longevity. They went on to show that the ability of MeNAM to extend life was dependent on its oxidation to its pyridone derivatives. This oxidation step generated a transient burst of hydrogen peroxide, which was the crucial signal for life span extension.[59] Whether caloric restriction induces increased longevity in much more complex mammalian species as it does in yeast and nematodes is a problem of much greater magnitude. In mice, caloric restriction was found to influence the expression of more than 3000 genes. Among these were ones involved in the mammalian target of rapamycin signal pathway and the *NNMT* gene. Overall, the change in the genetic expression profile in the liver male mice was found to gravitate towards that found in female liver. This more female expression was suggested to be the mechanism for the increased longevity.[60] That MeNAM may play a role in human longevity is uncertain. Oxidative free radicals are generally regarded as being disadvantageous in most instances, particularly in the brain. In a mouse model of Alzheimer disease, oxidising free radicals were seen to be part of the disease process. Nicotinamide, acting as a reducing agent, mitigated the action of the reactive oxygen species.[61] Whether NNMT and the subsequent conversion of NAM to MeNAM would be beneficial is uncertain. Ageing effects on other organs apart from the brain will have significant implications for longevity also. In the human kidney, ageing was found to increase the expression of STAT1 and STAT3 transcription factors. Signal transducer and activator of transcription 3 is a potent regulator of NNMT expression, as described below,[62] and NNMT is strongly expressed in the kidney. Thus, numerous organs, in addition to the brain, may undergo NNMT-modulated ageing, but whether caloric restriction really does influence our ageing is far from clear.

NNMT in NAM Catabolism and Phase 2 Catabolism of Xenobiotica

Nicotinamide *N*-methyltransferase acts as the major catabolic route for NAM to be excreted from the body. The conversion product MeNAM is excreted in the urine or is further oxidised to either the 1-methyl-2-pyridone 5-carboxylamide or 1-methyl-2-pyridone 3-carboxylamide derivatives by aldehyde oxidase, and these products are then excreted in the urine.[63–65]

The conversion of NAM to the MeNAM cation led to NNMT being classified as a component of the phase 2 clearance system for xenobiotica[66] because the positively charged ions formed are more easily excreted than the parent compound. Studies on animal NNMTs indicate that the enzyme has a broad substrate range. Mostly, data from studies on activities of nonhuman species have been assumed to apply to the human enzyme.[67]

The actions of phase 2 enzymes are thought to be beneficial, but this may not always be the case. An example of this is the possible action of NNMT in the central nervous system (CNS), in which the enzyme might convert some endogenous amines to toxic charged compounds that do not cross the blood-brain barrier easily to pass back into the peripheral circulation. In accordance with this concept of NNMT being capable of methylating a wide range of substrates, we have shown it to be able to methylate the nitrogen atom in position 2 of the naturally occurring β-carboline, norharman.[68] (It is very likely that NNMT was responsible for the *N*-methylation of pyridine in 2 human subjects reported by Caldwell et al.[69]) The specificity constant (k_{cat}/K_m) for norharman was lower than that of NAM, suggesting that NAM is the preferred substrate for NNMT.[68]

In contrast, these reactions occurring within the liver form charged compounds which are excreted or further metabolised and then excreted. Any cation so formed is unlikely to cross the blood-brain barrier and enter the CNS from the peripheral circulation, and so, the cations are relatively harmless. Methylation of these 2 compounds in the brain has an unfortunate result, however, in that the compounds formed are neurotoxins and they have been suggested to be linked to the Parkinson disease because of their ability to inhibit mitochondrial complex I.[70] Nevertheless, the specificity constant and catalysis constant (k_{cat}) for norharman are 54-fold and 256-fold lower than for NAM, respectively, suggesting that any *N*-methylation, particularly in the presence of NAM, will be very low. In addition, in vitro studies have demonstrated that 2-methylnorharman toxicity is significantly lower than that of its parent compound,[68] casting doubt on the possibility that it is a factor in the cause of the Parkinson disease.

NNMT as a Regulator of the Actions of NAM and NAD⁺/H

One of the most important functions of NAM is the formation of NAD⁺, which raises the question of what influence, if any, NNMT has on NAD⁺ availability. To address this question, the formation of NAD⁺ and its uses are discussed below.

Nicotinamide, from either the diet or the actions of a variety of cellular enzymes, enters the so-called salvage pathway for the formation of NAD⁺. In this, nicotinamide phosphoribosyltransferase (NAMPT) converts NAM to nicotinamide mononucleotide (NMN), using phosphoribosylpyrophosphate as the phosphoribotide moiety donor, and then 1 of the 3 forms of nicotinamide mononucleotide adenosyltransferase (NMNAT) converts NMN to NAD⁺, using ATP as the nucleotide donor (Figure 2). Nicotinamide mononucleotide adenosyltransferase 1 is located in the nucleus, and NMNAT2 is present in cytoplasm supplying those sites with NAD⁺. The origin of mitochondrial NAD⁺ is uncertain. Nicotinamide mononucleotide adenosyltransferase 3 has been suggested as the enzyme converting NMN to NAD⁺ in the mitochondrion,[71] but this is disputed.[72] An alternative explanation for the presence of NAD⁺ in the mitochondrion discussed by Yamamoto et al[72] is that of the action of hitherto unidentified transporters of the dinucleotide from cytoplasm into the mitochondrial matrix.

Once formed, in the mitochondrial matrix, NAD⁺ is converted to reduced nicotinamide adenine dinucleotide (NADH) to either serve as the electron and hydrogen ion donor for complex I or act as the cofactor for enzymes, such as silent information regulator 3 (sirtuin 3).[73] On leaving the mitochondrion, it may be converted into reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the cytoplasm, or it acts as substrate or cofactor for several enzymes in other cellular compartments and extracellular enzymes (Figure 2). A list of enzymes that consume NAD⁺ are as follows:

1. CD38 and CD157 (adenosine diphosphate [ADP]-ribosyl cyclase 2) are located on the plasma membrane and convert NAD⁺ to cyclic adenosine diphosphate ribose, the latter acting as a second messenger involved in calcium regulation.[74]
2. Adenosine diphosphate-ribosyltransferases may be divided into 2 groups. One group transfers a single ADP-ribosyl group (a process called MARylation),[75] eg, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin poly(ADP-ribose) polymerase, also known as ARTD14.[76] The second group comprises several enzymes termed poly ADP-ribosyltransferases. As the name signifies, they transfer multiple ADP-ribosyl groups (PARylation). They are involved in a variety of cellular processes,[77] eg, DNA repair and transcription elongation.[78,79]
3. Sirtuins, a group of 7 proteins located in cytoplasm (sirtuins 1 and 2), the mitochondrion (sirtuins 3, 4, and 5), or the nucleus (sirtuins 1, 2, 6, and 7). Most of the sirtuins deacetylate proteins such as histones involved in the regulation of gene expression.[80] Some deacylate other groups such as malonyl, succinyl, and glutaryl residues (sirtuin 5) or long-chain fatty acid residues (sirtuin 6). Although deacetylation of histones is a prominent feature of sirtuin action, their actions are not limited to this. They take part in a variety of intracellular reactions, eg, MARylation and autophagy.[75,81] Sirtuins influence a wide variety of cellular processes. These include the following:

   (a) Sirtuin 1 – mitochondrial biogenesis, ageing, and related diabetic complications[82,83];
   (b) Mitochondrial sirtuins 3, 4, and 5 – the response to oxidative stress, cell cycling, cell viability, and energy homeostasis in liver and heart;[84–86]

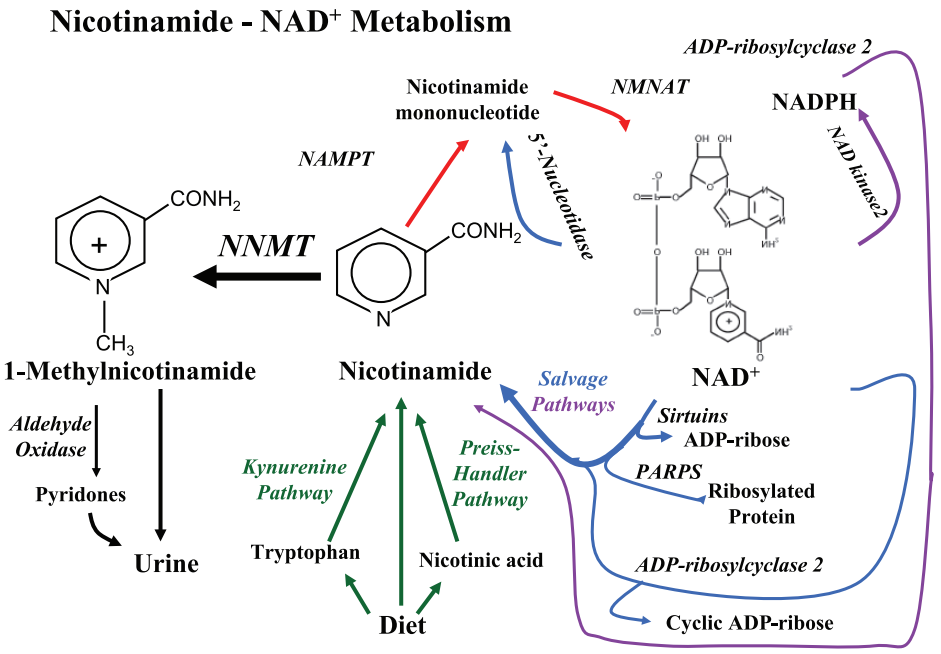

Figure 2. Metabolic pathways of nicotinamide. Arrows: green denotes entry of nicotinamide from diet; red, formation of NAD^+; blue and purple, salvage pathways to regenerate nicotinamide and nicotinamide mononucleotide; black, nicotinamide catabolic pathways. ADP indicates adenosine diphosphate; NAD^+, oxidised nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NMNAT, nicotinamide mononucleotide transferase; NNMT, nicotinamide *N*-methyltransferase; PARPs, poly ADP-ribosyltransferases.

(c) Sirtuin 6 – ageing[87];
(d) The NADH diphosphatase, NUDT12, located in peroxysomes,[88] converts NAD^+ to NMN which can then exit the organelle and enter the salvage pathway and in doing so contributes to the NAM pool.

The references mentioned above are mostly detailed recent reviews of the specific actions of the enzymes listed. More general reviews of the actions of these intracellular enzymes and their effects on cellular processes have been written by Opitz and Heiland,[89] Verdin,[90] and Nikiforov et al.[91] The consumption of NAD^+ and the liberation of NAM by the enzymes are illustrated in Figure 2. Interestingly, NAM acts as a potent inhibitor of some of these reactions.

The enzymes performing the multifarious reactions cited above are all likely to have their own normal ranges of expression in the various cells of the body and thus different rates of NAD^+ consumption. Given also that there are differing ranges of NNMT expression in cells, the individual organ contributions to MeNAM serum and urine concentrations are smoothed and do not reflect NAM metabolism in any specific organ. To a degree, giving a predetermined dose of NAM to temporarily fasted subjects overcomes the influence of diet on serum and urine measurements; however, complete 24-hour urine collections are notoriously difficult to obtain. Clearly, biopsy material would be ideal for assessing NAM metabolism in specific organs, but this is not readily available in healthy individuals. However, in disease states, both serum and tissues are available, and in these conditions, NNMT often does play an important in modulating NAD^+ usage.

Humans on a healthy diet might be expected to have sufficient vitamin B_3 in its various forms to ensure that NNMT is not the rate-determining factor in the formation of NAD^+/H for the above reactions. Nevertheless, the fact that NNMT has a wide expression range will influence an individual's daily requirement for the vitamin, with high expressors of NNMT presumably requiring a higher intake than low expressors. From this, it can also been seen that a suboptimal daily intake of niacin, as reported for a significant percentage of the general population of people aged more than 65 years in the United Kingdom,[92] could have serious health implications, as reported for congenital heart defects in children.[30]

NNMT and Energy Regulation

The role of NNMT in energy regulation has been studied in most detail in the hepatocyte and the white fat cell. In both instances, it is the effects on mitochondrial generation of energy, the major source of ATP in most cells, that are key, rather than glycolysis being key. The NADH is the electron and hydrogen donor for complex I; thus, varying levels of NNMT have the potential to alter levels of NAM and hence NADH directly. Nevertheless, for healthy individuals eating a good diet, vitamin B_3 intake is probably sufficient to ensure that the influence of genetically determined NNMT expression on NAM catabolism is not a major factor. In undernourished children, however, NNMT expression is thought to increase. NNMT expression is an adaptive response to the inadequate diet. This further reduces NAM availability for energy production. As a consequence, MeNAM excretion in the urine is increased. Brazilian children with high MeNAM

urinary excretion were found to have a better catch-up growth on refeeding than those with lower MeNAM excretion. It was posited that the high urinary MeNAM excretion had allowed these children to make a better adaptive response to the poor diet.[93]

The regulation of energy metabolism is subtle and needs to respond to demand and variable input from diet as well as the constant effect of genetics. Recent evidence has shown that sirtuins 1 and 3 are the major regulators of mitochondrial energy metabolism and, importantly, that NNMT is central to their actions. Factors regulating response of sirtuin 1 to diet, mediated by NAMPT effects on NAD^+ synthesis and the actions of transcription factors, such as forkhead box protein O (FOXO)-1, -3, and -4, were reviewed by Chalkiadaki and Guarente[94] in 2011. The following year, actions of sirtuins 1 and 3 were reviewed in more detail by Nogueiras et al,[95] although any role for NNMT was not considered in these reviews. Subsequently, Kraus et al described the effects of NNMT knockdown on mice and a high-fat diet on white fat cell metabolism. They showed that a high-fat diet increased NNMT expression. When they knocked down NNMT expression using antisense oligonucleotides, they found that

> "NNMT knockdown in adipose tissue and liver protected mice from diet-induced obesity, causing a 47% reduction in relative fat mass and a 15% increase in relative lean mass."

These changes were due to increased energy expenditure (increased oxygen consumption) rather than reduced food intake. This action resulted from the effect of NNMT expression on polyamine flux. Kraus et al postulated that in the white cell, decreased NNMT expression allowed more SAM to be 'shunted' into polyamine metabolism in which polyamines are acetylated and then excreted into the urine for further catabolism, thus increasing energy expenditure. The levels of NAD^+, the cofactor of sirtuin 1, were increased, allowing increased expression of the target genes of sirtuin 1. Increased NAD^+ was also thought to favour increased ATP synthesis, thus allowing a further increase in energy expenditure.[96]

In accordance with these findings on the deleterious effects in the white fat cell, NNMT expression was found to be upregulated in both subcutaneous and omental white fat cells of patients with type 2 diabetes. Kannt et al analysed cells from 55 women and 33 men with type 2 diabetes and compared the results with those from 73 women and 38 men without the disease. Generally, women tended to have higher results than men, and when either of the diabetic sex's results were compared with the appropriate nondiabetic sex's results, they were found to be significantly higher. Plasma MeNAM concentration correlated with total white fat NNMT expression in patients with diabetes but not in nondiabetic patients.[97] In a larger study involving 691 men and 469 women, serum MeNAM concentration was found to correlate significantly positively with body mass index (kg/m^2), waist circumference, hip circumference, and low-density lipoprotein (LDL) cholesterol and negatively with high-density lipoprotein cholesterol. Serum MeNAM concentration was associated with a high risk of obesity (N = 1160, odds ratio of 3.04 with 95% confidence interval of 1.61–5.73, and significance of $P < .001$), further emphasising the relationship of NNMT and MeNAM with white fat cell metabolism.[98,99]

In contrast to what happens in the white fat cell, in the liver increased NNMT expression was found to have a beneficial effect. Again, this was shown by Hong et al[100] to occur via the effects of NNMT action on sirtuin 1 activity. Prior to the paper of Hong et al, increased sirtuin 1 activity was known to upregulate gluconeogenesis[101–103] and suppress cholesterol synthesis and lipogenesis.[104,105] Using mice and mouse hepatocytes, Hong et al showed that MeNAM mediated all the effects of NNMT by stabilising hepatocyte sirtuin 1 protein, so decreasing its catabolism and increasing its half-life, without any effect on sirtuin 1 gene transcription. Increased NNMT leading to increased sirtuin 1 activity resulted in increased glucose output from hepatocytes, and ablation of NNMT synthesis resulted in the opposite effects. Importantly, Hong et al showed that their mouse in vivo and hepatocyte in vitro results were mirrored in humans. They assayed the NNMT mRNA extracted from liver biopsy samples by reverse transcription polymerase chain reaction (RT-PCR); serum cholesterol, LDL cholesterol, triglycerides, and glucose by routine clinical biochemical assays; and insulin activity by euglycaemic, hyperinsulinaemic clamp methodology in samples from 51 obese patients. Hepatic NNMT mRNA levels were shown to be significantly positively correlated with glucose infusion rate and serum cortisol and significantly negatively correlated with serum total cholesterol, LDL cholesterol, and total fasting triglycerides.[100]

The role of sirtuin 3 in energy metabolism has been reviewed in detail by Nogueiras et al[95] in 2012. The central role of sirtuin 3 in the modulating response of hepatocyte mitochondria has been further explored by Liu et al.[106] They studied the effects of fasting in mouse hepatocytes and found that fasting induced pronounced transcription of mitochondrial oxidative phosphorylation (OXYPHOS)-related genes, with relatively little effect on the transcription of nuclear DNA–encoded genes. This induction was independent of mitochondrial neogenesis. Furthermore, they showed that sirtuin 3 was 'necessary and sufficient' to mediate this induction. They showed that fasting induced glucagon secretion and proposed that this gave rise to increased mitochondrial NAD^+, which is the cofactor for sirtuin 3, allowing increased sirtuin 3 activity. The origins of mitochondrial NAD^+ are of some debate, but any reduction in cytosolic NNMT would increase the availability of NAM for NAD^+ synthesis, thus implicating NNMT in sirtuin 3's actions.

Another potential site of NNMT's action on energy metabolism is the brain. Nicotinamide *N*-methyltransferase is expressed in various parts of the CNS, which also expresses sirtuins 1 and 3. Nevertheless, the role of NNMT and

MeNAM in the normal healthy brain is unclear. Nicotinamide *N*-methyltransferase is increased in the Parkinson disease,[34,107] and MeNAM has been shown to ameliorate some of the effects of diabetes in the brain in a rat model,[108] prompting the idea that the increase in NNMT, and consequently MeNAM, is a defensive response to protect neurons. The topic of NNMT and energy production is clearly complex. One article comments on NNMT as bad in fat and good in liver.[109] This complexity is increased by the interplay between the mitochondrion and the nucleus in response to nutrient availability and stresses of various nature.[110,111] Nicotinamide *N*-methyltransferase is not always considered as a participant in this scenario, although it clearly does have a role. Only further research will elucidate this role.

Expression and Actions of NNMT and MeNAM in Cancer

The description of overexpression of NNMT in human thyroid cancer by the group of Hershman was the first detailed report of this phenomenon in cancer.[112] This study has been followed by reports of the enzyme's generally increased expression in a wide range of cancers, with the group of Emanuelli being responsible for a significant number of such reports. The range of cancers involved is shown in Table 1. Nevertheless, it should be remembered that this body of work is a corollary of earlier work showing the link between increased MeNAM excretion and cancer.[131–133] As a phenomenon, the increased production of NNMT in this first case prompts 4 questions: (a) is it elevated in every cancer, (b) what causes its expression to be deregulated, (c) what is the consequence of its deregulation, and (d) what use can be made of the phenomenon itself and the facts revealed in the answers to questions (a) to (c).

The reasons for the deregulation of NNMT expression in cancer are far from clear. Cancers have been postulated to arise from transformed stem cells, and the process of transformation is one of dedifferentiation. Interestingly, NNMT is said to modulate the epigenetic environment in the differentiation process in stems cells in the earliest period of human life.[51] Also, in 1 neoplasm, NNMT expression was found to be higher in stem cell–enriched cultures of Hep-2 cells (originating from cancer of the larynx) compared with the level in cultures of the parental cells.[134] In view of the above, the increased levels of NNMT in cancers may be regarded as playing a role in changing DNA methylation status, thus favouring a pattern of gene transcription more in tune with a dedifferentiated state. In addition, 2 of the transcription factors known to regulate NNMT expression, STAT3 and TGF-β_1 (Figure 3), are highly expressed in numerous cancers, including clear cell renal cell carcinoma and oral squamous cell carcinoma.[125,135] However, NNMT expression is not always elevated, as will be shown below. Thus, such a simple explanation is difficult to hold. Therefore, in an attempt to clarify the ups and downs of NNMT expression, the succeeding paragraphs describe factors influencing NNMT expression and what are thought to be some of the consequences of its expression.

As can be seen from Figure 2, NNMT stands juxtaposed to the cascade of NAD^+-forming and NAD^+-consuming enzymes, forming the major catabolic route for NAM. The formation of MeNAM in this catabolic route has often been thought to be the only function of NNMT, with MeNAM serving as an inert, more easily excreted product compared with NAM. However, this would be to ignore data evidencing the cell growth–promoting properties of MeNAM,[131–133] which link to earlier data suggesting a relation between NAM methylation and cancer.[136–138] Nevertheless, even if the biologically inert nature of MeNAM were the case, supranormal expression of NNMT would have the capability to not only influence NAM, NAD^+/H, and NADPH levels but also those of SAM, and ultimately homocysteine. Thus, NNMT has been envisaged as a metabolic sink, its high expression in many cancers resulting in lower availability of SAM, as judged by the SAM:SAH ratio, and hence hypomethylation of histones.[139] (The SAM:SAH ratio reflects the ability to methylate DNA at cytosine residues.) This epigenetic modification of DNA (methylation) tends to downregulate gene transcription.[140] The high expression of NNMT in cancers may also limit the availability of NAM and NAD^+ for participation in reactions other than DNA methylation, which are illustrated in Figure 2. Thus, coupled with changes in the pattern of DNA methylation from effects on SAM availability, the effects of increased expression NNMT on NAD^+ availability and consequently on the actions of sirtuins on histone deacetylation and energy metabolism will favour carcinogenesis. The above actions might be relatively unimportant if NNMT expression was high in only a limited number of rare cancers, but this is not the case. Nicotinamide *N*-methyltransferase is highly expressed in a wide range of cancers, as illustrated in Table 1. This shows that NNMT expression is elevated in cancers in several different areas of the body and includes most of the common human malignancies. The question then arises as to what part NNMT plays in the process of carcinogenesis. The possibilities of the increased NNMT are as follows: a cause of carcinogenesis, a supportive consequence of carcinogenesis, an irrelevant consequence, and an anticarcinogenic reaction of the cell. The available data tend to favour the second option – a consequence supportive of carcinogenesis – because of increased NNMT and MeNAM support:

1. Increased mitochondrial ATP synthesis[141,142];
2. Increased cell replication[143];
3. Increased cell migration and metastatic invasion of tissues.[144,145]

The following paragraphs concentrate on the effects of increased expression because this is the more common phenomenon, but it should be remembered that this does not occur

Table 1. NNMT expression in cancer.

CANCER	CANCER TYPE	AUTHORS	NUMBERS	TISSUE/FLUID	COMPARISON	ANALYTE AND RESPONSE	METHODS
Bladder		Riester et al[113]	93	Cancer	Published data	mRNA +ve	cDNA array
		Sartini et al[114]	28	Cancer	28 self normal tissue	mRNA and protein +ve	RT-PCR Western blot activity
Breast	Triple-negative cancer	Kuo et al[115]	51	Cancer	106 luminal breast cancer	mRNA +ve	cDNA array
Colorectal		Roessler et al[116]	16	Cancer	16 self normal tissue	Protein +ve	Electrophoresis/mass spec.
		Roessler et al[116]	109	Serum	317 healthy controls	Protein +ve	ELISA
		Tomida et al[21]	88	Cancer	17 healthy controls	Protein +ve	Immunohistochemistry
Gastric		Chen et al[117]	641	Cancer (formalin fixed)	94 self normal	mRNA	Tissue microarray
Glioma	Glioblastoma rich	Li et al[118]	90	Cancer	69 oligodendroglioma-rich and published data	mRNA +ve	cDNA array and unsupervised machine learning
Hepatocellular carcinoma		Kim et al[119]	120	Cancer	Cancer stages	mRNA –ve	RT-PCR
Insulinoma		Nabokikh et al[22]	9	Cancer	4 pancreatic cell preparations	mRNA –ve	RT-PCR
Lung	Non–small cell	Sartini et al[120]	36	Cancer	Adjacent tissue	mRNA +ve	RT-PCR
		Tomida et al[42]	113	Serum	50 non-neoplastic lung disease	Protein +ve	ELISA
Lymphoma	Peripheral T-cell lymphoma, unspecified	Piccaluga et al[121]	28	Cancer	20 normal T cells	mRNA +ve	cDNA array
	Angioimmunoblastic	Piccaluga et al[122]	6	Cancer	28 peripheral T-cell lymphoma, unspecified	mRNA +ve	cDNA array

Table 1. (Continued)

CANCER	CANCER TYPE	AUTHORS	NUMBERS	TISSUE/FLUID	COMPARISON	ANALYTE AND RESPONSE	METHODS
Nasopharyngeal carcinoma		Win et al[123]	124	Tissue	Cancer stages	Protein +ve	Immunohistochemistry
Oral carcinoma	Oral squamous cell	Sartini et al[124]	22	Tissue	Self normal tissue	mRNA and protein +ve	RT-PCR Western blot
Oral carcinoma		Emanuelli et al[125]		Tissue	Cancer stages	Protein	Immunohistochemistry
		Jiang et al[126]		Affymetrix microarray data gse31853	Normal tissue	mRNA and protein +ve,	Bioinformatics
Ovarian carcinoma	Ovarian serous papillary carcinoma, metastatic	Bignotti et al[127]	17	Metastatic cancer tissue	14 primary cancer tissue	mRNA +ve	Oligonucleotide microarray
Pancreatic cancer		Rogers et al[128]	6	Pancreatic juice	10 non-neoplastic	mRNA +ve	Oligonucleotide microarray
		Xu et al[129]	178	Cancer tissue	28 chronic pancreatitis	Protein +ve	Immunohistochemistry
Prostate cancer		Zhou et al[130]	120	Cancer tissue	26 BPH 18 HGPIN	+ve	
		Heemers et al[23]		Cancer tissue	Androgen-responsive genes	mRNA +ve	cDNA microarray Ingenuity Pathway Analysis
Thyroid	Papillary	Xu et al[112]		Cell lines		mRNA Protein +ve	RT-PCR Northern blot Western blot Catalytic activity
		Xu et al[20]		Cell lines		mRNA	RT-PCR

Abbreviations: BHP, benign prostatic hyperplasia; cDNA, complementary DNA; ELISA, enzyme-linked immunosorbent assay; HGPIN, high-grade prostatic intraepithelial neoplasia; mass spec., mass spectrometry; mRNA, messenger RNA; NNMT, nicotinamide *N*-methyltransferase; RT-PCR, reverse transcription polymerase chain reaction.
Normal tissue means tissue taken from individuals without cancer; self normal tissue, healthy tissue taken from the same patient as the cancer tissue; cancer stages, tissue taken from cancers of different stages to compare with one another; +ve, upregulation; –ve, downregulation.

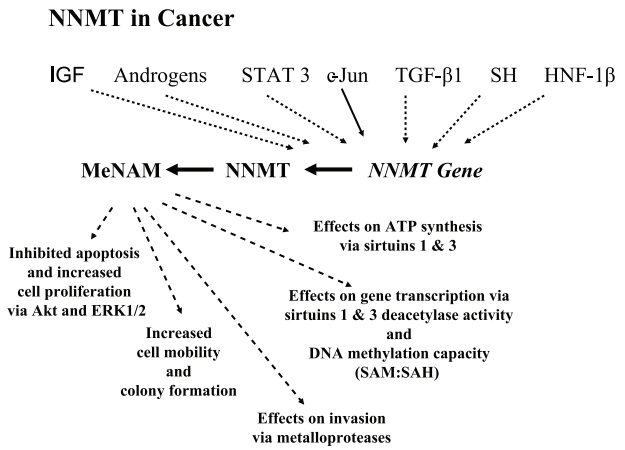

Figure 3. Factors influencing NNMT expression and results associated with NNMT expression in cancer. Akt indicates protein kinase B; c-Jun, Jun transcription factor; ERK1/2, extracellular signal–regulated kinases 1 and 2; HNF-1β, hepatic nuclear factor-1β; IGF, insulin-like growth factor; MeNAM, 1-methylnicotinamide; NNMT, nicotinamide *N*-methyltransferase; SAH, *S*-adenosyl homocysteine; SAM, *S*-adenosyl methionine; SH, sonic hedgehog; STAT3, signal transducer and activator of transcription 3; TGF-β_1, transforming growth factor β_1.

in every cancer. Furthermore, it does not occur in every individual patient, even though in a group of such cases, its median level of expression is elevated.

Increased NNMT Expression and ATP Synthesis in Cancer

To illustrate point (1), we transfected NNMT into SH-SY5Y cells (human neuroblastoma) which do not express the enzyme. The transfected cells had significantly decreased cell death compared with that seen in wild-type cells. This decrease was correlated with increased intracellular ATP content, ATP:ADP ratio, and complex I activity and a reduction in the degradation of the NDUFS3 (NADH dehydrogenase (ubiquinone) iron-sulphur protein 3) subunit of complex I. These effects were replicated by incubation of SH-SY5Y cells with MeNAM, strongly suggesting that MeNAM mediated the cellular effects of NNMT. Furthermore, expression of NNMT in SH-SY5Y cells significantly increased the expression of sirtuins 1, 2, and 3. Small interfering RNA–mediated silencing of sirtuin 3 expression decreased complex I activity in NNMT-expressing SH-SY5Y cells to that observed in wild-type SH-SY5Y and significantly reduced cellular ATP content.[141,142] Although in vivo cancer cells undergo a shift away from mitochondrial OXYPHOS and towards glycolysis as their source of ATP,[146,147] the presence of higher than normal levels of MeNAM may help to maintain a level of OXYPHOS in the changed environment of rapidly dividing cells. In view of the fact that mitochondria are generally similar throughout the human body, this effect of MeNAM on OXYPHOS may occur in all types of cancer in which increased expression of NNMT occurs. Whether the result of high NNMT expression is benign is less certain. Although OXYPHOS was found to be higher in PANC-1 cells expressing the enzyme compared with ones not doing so, the NNMT-expressing cells were more resistant to glycolytic inhibition and they had greater cell migration and invasion capacities.[148] These findings contrast with the findings of Capello et al[147] who found that silencing alpha enolase expression restored OXYPHOS and induced cell growth arrest in a variety of cancer cell lines.

In contrast to the idea that the level of NNMT expression is always increased in cancer, Yamada et al explored the effect interferon gamma on NNMT in glioma. Treating the cancer with interferon gamma resulted in increased NNMT activity and hence MeNAM levels, but not NNMT expression. They also treated these cells with MeNAM. Neither treatment affected intracellular NAD^+ levels or cell viability. As a consequence, they questioned the idea that increased NNMT in cancer always reduced cellular NAM concentration in tumour cells.[149]

Increased Expression of NNMT Generally Favours Increased Cell Replication, Cell Migration, and Metastatic Invasion

The evidence for NNMT and MeNAM increasing cell proliferation is mixed. Early reports indicate that MeNAM supports cell replication.[131–133] In addition, some recent authors report data that support the contention. Later data come from transfection experiments using model cell systems. Thus, Yu et al[148] state that NNMT overexpression in PANC-1 cells (a pancreatic tumour cell line) promoted cell proliferation, whereas NNMT knockdown with silencing mRNA reduced proliferation. Xie et al[150] using colorectal cells (human colorectal cancer cells line SW480), Zhang et al[151] using Bcap-37 and MDA-MB-231 breast cancer cell lines, and Sartini et al[120] using lung cells (human lung cancer cell line A549) reported similar findings. Wu et al conducted a survey of results from 40 human bladder cancer cell lines to identify factors influencing cell migration. The results were used to assay tumour stage in 61 patients. Together, these analyses identified metallothionein 1E and NNMT as being significantly positively correlated with cell migration and tumour stage.[145]

Regarding other markers of tumorigenicity, Xie et al[150] reported that NNMT overexpression enhanced colony formation in vitro and tumorigenicity in mice and inhibited apoptosis and promoted cell cycle progression, MeNAM alone producing similar results. Knockdown of NNMT in the lung cell model had the reverse effect, in that colony formation was inhibited.[120] Similarly, reduction in the expression of NNMT in breast cancer cells caused reduced expression of Bcl-2 and Bcl-xL and decreased phosphorylation of protein kinase B (Akt) and extracellular signal–regulated kinase 1/2 (ERK1/2). Together with the upregulation of Bax, Puma, cleaved caspase 9, cleaved caspase 3, and cleaved poly ADP-ribosyltransferase, these data indicated the downregulation of NNMT-induced apoptosis via the mitochondrial pathway.[151]

Further evidence of the involvement of the phosphoinositide 3-kinase/Akt pathway in tumorigenicity comes from the work of Wu et al. They showed that there were positive correlations between NNMT and NNMT and matrix metallopeptidase-2 (MMP-2) in clear cell renal cell carcinoma cell lines and clinical tissues.[145] Matrix metallopeptidase-2 is a member of a family of 23 such enzymes which are responsible for turnover of extracellular matrix by proteolytic degradation.[152] Inhibition of MMP-2 significantly suppressed NNMT-dependent cellular invasion in HEK293 cells. In vivo 'knockdown of NNMT expression efficiently inhibited the growth and metastasis of clear cell renal cell carcinoma cells in non-obese diabetic severe combined immunodeficiency mice'.[144]

In contrast to the above, the effects of increased MeNAM and NNMT on cancer are more complex than simply increasing proliferation and turmorigenicity. In fact, their effects differ depending on the cancer. Hence, Sartini et al[124] report that in oral squamous cell carcinoma, NNMT upregulation correlates inversely with lymph node metastasis. This was followed by reports from the same group that (a) upregulation of the enzyme correlated positively with the differentiation state of the cancer[125] and (b) knockdown of the enzyme reduced proliferation in the human oral squamous cell carcinoma cell line, KB.[153] Zhou et al also reported data that support the concept that NNMT expression in not necessarily deleterious. They found that NNMT was highly expressed in only 64.2% (77 of 120) of their cases, but as high as 83.3% in high-grade prostatic intraepithelial neoplasia, and that NNMT expression was a positive prognostic indicator of survival in prostate cancer.[130]

In accordance with the findings on oral squamous cell carcinoma, Blazejczyk et al report that MeNAM and its analogue, 1,4-dimethylpyridine, inhibited metastasis formation in the lung in a mouse model of mammary cancer. They showed that this effect of the 2 compounds was enhanced when they were administered with cyclophosphamide. They proposed that MeNAM acted via significantly increasing prostacyclin GI_2 activity.[154]

Factors Influencing and Influenced by NNMT Expression in Cancer

Four important transcription factors have been shown to have a significant role in modulation of NNMT expression. These are hepatocyte nuclear factor-1β in papillary thyroid cancer,[20] STAT3 in colon adenocarcinoma,[21] and sonic hedgehog ligand in pancreatic cancer.[155] In contrast to these 3 examples, where NNMT is elevated, the reduction in NNMT expression in insulinoma cells was shown by Nabokikh et al[22] to be correlated with reduced TGF-β_1 expression. A detailed example of this relationship between a transcription factor and NNMT expression is explained below:

> "We observed there was enhanced expression of NNMT in cytoplasm of 76 (86%) colon adenocarcinoma tissues among 88 evaluable samples. On the other hand, 16 of 17 normal colon tissues were weakly stained with NNMT antibody by immunohistochemistry. Of the 88 cancer tissues, 66 (75%) were positive for phosphorylated STAT 3 (tyrosine 705) antibody, whereas all normal tissues were almost negative. Of the 66 nuclear phosphorylated STAT 3–positive cases, 61 (92%) were also strongly stained with anti-NNMT antibody. These results showed that enhanced expression of NNMT was correlated with activation of STAT 3 ($P < 0.001$)."[21]

Clearly, all 4 of these factors are unlikely to be acting in all cancers, and other transcription factors may well be acting in cancers other than those cited above. Nevertheless, the important feature of the increased expression of NNMT in most cancers is most likely due to increased transcription rather than any effect on catabolism. The reduced expression in insulinoma and colorectal carcinoma would seem to add support to this idea. Furthermore, many of the effects of increased NNMT expression appear to be mediated by increased synthesis of MeNAM.

Another factor influencing expression in a cell model was investigated by Bi et al. They explored the relationship between PANC-1 cells expressing a gain-of-function noncoding microRNA-1291 (miR-1291) and control PANC-1 cells. The former cells expressed significantly higher mounts of NNMT and MeNAM than control cells. In a xenograft model where nude mice were injected with either miR-1291 PANC-1 or control PANC-1 cells, NNMT expression was greater in the animals receiving the miR-1291 PANC-1 cells, and tumour size and weight were less, resulting in NNMT levels being inversely correlated with tumour size.[156]

Other Effects of Increased NNMT Expression

One of the consequences of increased NNMT expression is reported to be increased resistance to the effects of radiation. This was reported to occur in cancer stem cells by D'Andrea et al[157] who studied 2 mesenchymal stem cell clones, one expressing NNMT and the other not, in a model of Ewing sarcoma. Ewing sarcoma is the second most common bone cancer in children. It is a high-grade malignancy in which 25% of patients are metastatic at presentation and which has a very poor prognosis. It grows rapidly and metastasises to lungs and bones. The NNMT-expressing cells were found to have greater resistance to radiation. D'Andrea[158] postulated that the increased NNMT activity resulted in lower intracellular NAM levels, thus limiting DNA repair by PARP1, which uses NAM as a cofactor. This complements earlier work by Kassem et al,[159] who found that NNMT helps to predict the response to radiation in bladder cancer. Knockdown of NNMT in a glioma cell line (U87) also rendered the cells more susceptible to radiation.[36]

Nicotinamide *N*-methyltransferase expression has been found to be related to drug resistance. Hsu et al[160] related the resistance of 78 cancer cell lines to 99 drugs and found that 8 genes (*EGFR, ITGA3, MYLK, RAI14, AHNAK, GLS, IL32*,

and *NNMT*) showed significant gene-drug correlation with paclitaxel, docetaxel, erlotinib, everolimus, and dasatinib. In contrast to the above, where NNMT expression has been related to resistance to therapeutic agents, 1 group reports a beneficial effect. Kwon et al studied the actions of sulphonamide analogues of antofine and cryptopleurine. They proposed that the inhibited growth of Caki-1 renal cancer cells seen after exposure to one of the sulphonamide cryptopleurine analogues occurred via the action of NNMT-dependent c-Jun N-terminal kinase activation on G_0/G_1 cell cycle arrest.[161]

NNMT as a Biomarker in Cancer

As can be seen from Table 1, NNMT is upregulated in a wide range of cancers, and the authors of these reports generally postulate the idea that NNMT could function as a valuable biomarker. Precisely, how NNMT assay might be used as a biomarker differs with each cancer. As a minimally invasive screening tool, serum or plasma NNMT concentration would be a sum of NNMT released by the cancer and that released into the circulation from the liver. Given that (a) NNMT expression in healthy livers is higher than in other organs and (b) most cancers are small compared with the liver, one might expect that NNMT release from the cancer might have little impact on circulating concentration. Circulating MeNAM concentration may have similar contributions from liver and the cancer in some circumstances, but there would be further variations because of differences in diet, making circulating MeNAM concentrations difficult to interpret in theory. Nevertheless, NNMT has been suggested as a biomarker in fluids, and where it has been suggested as such is reviewed below.

As an identification, classification, and prognostic tool, the assay of NNMT in the cancer tissue compared with that in nonmalignant tissue, in theory, offers more promise than blood and other biofluid concentrations. Although NNMT expression is upregulated in many cancers, it is not upregulated in all cancers. To address the problems associated with the use of NNMT as a biomarker in tissues, the literature concerning the following 4 cancers is reviewed in detail: renal cell carcinoma, glioma, oral squamous cell carcinoma, and hepatocellular carcinoma. Other cancers where deregulation of NNMT expression occurs are summarised in Table 1.

The following sections review the latest data on the use of NNMT and MeNAM as biomarkers in serum and plasma and in tissue samples. These sections are placed in the context of early data on these topics.

Early Evidence of Deregulation of NNMT

There are 2 early reports of the quantification of MeNAM in cancer or cancer-related activity – by Kerr et al in 1965 and Basu et al in 1973. Kerr et al[162] reported that smoking caused an approximately 30% fall in MeNAM excretion, and excretion rose on cessation of the activity. Basu et al found that MeNAM excretion rates in their cancer patients (mean 2.7 mg/g creatinine; range: 0.4-3.0, N = 58) were significantly lower than those of healthy patients (mean 9.0 mg/g creatinine; range: 3.8-11.2, N = 15) and those of patients who were ill due to nonmalignant causes (mean 8.1, range: 2.1-18.47 mg/g creatinine, N = 18).[163] Their data indicate that NNMT activity was reduced in patients with cancer, probably due to their extensive treatment. These reports contrast with later ones describing patients at presentation and were made before efficient NNMT antibodies, which allow accurate assay of the enzyme's expression, became available.

NNMT as a Diagnostic and Prognostic Biomarker – Use of Biofluids

Roessler et al used 2-dimensional electrophoresis and protein mass spectrometry to identify proteins which were differentially expressed in colorectal cancer tissues compared with levels in normal bowel tissue. One of the proteins identified was NNMT. From there, they developed an immunoassay for the protein. In the serum samples of normal healthy individuals (N = 317), they reported a median NNMT concentration of 308 ng/L and a range of 146 to 2625 ng/L compared with that of 109 patients with cancer, who have a median NNMT concentration of 925 ng/L and a range of 169 to 2625 ng/L. They postulated that serum NNMT concentration was a promising biomarker for colorectal cancer.[116] In contrast, Bünger et al[164] also compared serum NNMT concentrations of 164 patients with colon cancer, 34 patients with adenoma, and 119 healthy subjects and found that serum NNMT concentration was not a particularly useful marker.

Tomida et al compared serum NNMT and carcinoembryonic antigen (CEA) concentrations as diagnostic biomarkers in patients with non–small cell lung cancer – 50 with non-neoplastic lung disease and 24 healthy donors – to evaluate the 2 protein concentrations as minimally invasive diagnostic assays. Concentrations were determined by enzyme-linked immunosorbent assays. Areas under the receiver operating characteristic curves for the 2 proteins were 0.703 for NNMT and 0.621 for CEA. With a specificity of 90%, the sensitivities for NNMT and CEA were 25% and 24%, respectively. There was no correlation between the CEA and NNMT concentrations, and combining the 2 parameters increased sensitivity.[42] In another study by Ujiie et al, although serum NNMT concentration was found to be elevated in non–small cell lung cancer, as a prognostic guide, serum NNMT proved not to be useful. They followed 107 patients over a period of 200 days and found that patients with NNMT concentrations less than 710 ng/L (N = 82) had similar survival rates compared with patients with serum concentrations equal or greater than 710 ng/L.[165]

Using a combination of NNMT, L-Plastin (LPC1), and nonmetastatic cell 1 protein (NM23A) plasma concentrations in a 3-marker assay for the diagnosis of renal cancer, Su Kim et al found that this combination had a sensitivity of 95.7% (specificity 90%) as opposed to 71.9% for NNMT alone. The diagnostic accuracy of the combination was 0.932 (positive

predictive value, 87.2%; negative predictive value, 97%). In these patients, median plasma NNMT concentration was 68 ng/L (95% range, 54.8-83.2; N = 102) for a control group of mainly healthy individuals compared with 420 ng/L (332.6-511.8, N = 87) for patients with renal cancer. When the performance of NNMT alone was compared with the 3-assay combination, the sensitivities were for clear cell carcinoma 93% (83 of 89 cases) compared with 96% (85 of 89 cases), for papillary carcinoma 100% (6 of 6 cases) compared with 100%, and for chromophobe carcinoma 86% (6 of 7) compared with 86%, the diagnostic cutoff level being 147 ng/L. When cancer staging was considered, the results for NNMT assay were similar to those of the combination.[166]

Elevated levels of NNMT in 4 other biofluids were identified and suggested as possible diagnostic and/or prognostic indicators. These were (a) urine for bladder cancer,[114] (b) pancreatic juice for pancreatic cancer,[128] (c) saliva for oral cancer,[167] and (d) interstitial fluid for clear cell renal cell carcinoma.[168] The numbers of patients investigated in these 4 studies were relatively small, making any assessment of assaying NNMT as a biomarker difficult.

It can be seen from the above that (a) the estimates of NNMT concentration in serum and plasma vary quite widely and (b) in patients with colorectal cancer the usefulness of serum/plasma NNMT estimation as a diagnostic tool is disputed. To clarify these diverse positions, a recognised standard NNMT preparation and multicentre trials are required.

NNMT as a Diagnostic and Prognostic Biomarker – In Tissue Samples

Renal cell carcinoma

Of all the investigations where NNMT in cancer is the primary topic, probably the most investigated cancer is renal cell carcinoma. Since 2005, following papers by Yao et al[169] and Jones et al,[170] a further 12 papers have been published on the subject of NNMT as a biomarker, with the authors using a variety of methodologies to explore expression in cancerous tissue. Yao et al using high-density oligonucleotide microarrays explored gene expression profiles of 33 renal cell carcinomas, the results of which were compared with those of 9 normal kidney tissue samples. Nicotinamide *N*-methyltransferase and adipose differentiation–related protein were found to be upregulated.[169] Using a different technique (immunohistochemistry), Zhang et al[171] examined one specific type of renal cancer (clear cell renal cell carcinoma, N = 74) and found that elevated NNMT levels were a significant prognosticator, high levels indicating a worse prognosis. Using yet another technique (mass spectrometry–based proteomic analysis, in which proteins separated by 2-dimensional electrophoresis are identified by mass spectrometry), Teng et al compared protein expression in clear cell renal cell carcinoma tissue with that of adjacent normal tissue. This comparison of tissues from the same patient confirmed that NNMT expression was elevated.[172] Similar results (high NNMT expression) were found by (a) Girgis et al[173] using multilevel whole-genome analysis, (b) Atrih et al[174] using quantitative proteomics, (c) Zaravinos et al,[175] and (d) Eikrem et al.[176] These last authors extracted mRNA from formalin-fixed tissues before performing transcriptome sequencing.

Using data from previous studies, Moffitt et al applied a bioinformatics approach to select the most reliable biomarkers for clear cell renal cell carcinoma. They claimed that assessment microarray data in the presence of artefacts using caCORRECT2 program gave results of greater accuracy and reliability. Their judgement was that *NNMT* was 1 of 2 genes that were the most reliable diagnostic indicator of clear cell renal cell carcinoma, the other gene being *PRKAB1*.[177] Use of the Ingenuity Pathway Analysis program to analyse data sets for genes deregulated in clear cell renal cell carcinoma revealed a number of transcription factor genes in the top 1% of genes deregulated.[175] Among these was the *STAT3* gene, a transcription factor known to influence NNMT expression.[21] A further development was that made by Wozniak et al. These authors compared the methylation status of the *NNMT* gene in clear cell renal cell carcinoma with that in adjacent normal tissue. Genome-wide gene expression profiling was performed on 148 clear cell renal cell carcinoma tissue samples from patients in the Czech Republic. Not only was NNMT mRNA levels found to be elevated but also the *NNMT* gene was found to be hypomethylated in the cancer tissue,[178] which would help to explain the increased expression of the protein. Interestingly, in an earlier report by Cifola et al[179] on increased incidences of loss of heterozygosity and variations in copy number, they found that alterations in CN occurred quite frequently in chromosome 11q22 region but not in the 11q23 region where the *NNMT* gene is sited. A similar result (ie, no genetic aberration in renal cell carcinoma) was reported subsequently by Arai et al.[180]

As an assessment tool in cancer staging NNMT was found to be modestly elevated in stages 1 to 3 of clear cell renal cell carcinoma; however, in stage 4 and in metastases, NNMT was highly elevated, in accordance with reports cited above of NNMT protein being involved in increased cellular reproduction and invasion by cancer cells.[181] When NNMT mRNA expression levels were assayed in 17 individual clear cell papillary renal cell carcinomas, 15 individual clear cell renal cell carcinomas, and 13 papillary renal cell carcinomas, clear cell renal cell carcinomas were found to have significantly higher levels than papillary renal cell carcinomas, with clear cell papillary renal cell carcinomas having levels that were between the other two.[182]

Alongside the interest in NNMT in clear cell renal cell carcinoma, NNMT features as a potential biomarker in several other cancers because of deregulated NNMT expression. The relevant papers are summarised in Table 1. Nevertheless, 4 other areas of interest are dealt with in some detail here. These are oral squamous cell carcinoma, prostate cancer, glioma, hepatocellular carcinoma, and insulinoma. In the first

two of these, NNMT is generally elevated, but in the last two, NNMT expression is reduced in comparison with nonmalignant tissue.

Glioma

Numerous attempts to identify and classify gliomas using wide-ranging transcriptome analysis techniques have been attempted; however, NNMT did not feature in the results in any of these until the report of Li et al in 2009. After examining both their own material (159 samples) and data from 2 external data sets (341 patients), they were able to distinguish 6 subgroups of gliomas. Although NNMT was only briefly mentioned even in this report, they concluded that NNMT was significantly more highly expressed (approximately 5-fold) in glioblastoma-rich groups compared with oligodendroglioma-rich groups.[118]

In accordance with the above finding in glioblastoma, Thirumoorthy et al[36] found that NNMT expression was higher in primary glioblastoma cell cultures than in established cell lines. Interestingly, although Yamada et al also confirmed the high levels of NNMT in glioblastoma, they found that the high levels of MeNAM had no effect on cell viability or on intracellular NAD+ and NAM concentrations. In addition, they observed that treatment with interferon gamma increased NNMT activity but not its protein concentration.[149] A further curiosity revealed by Kiprianova et al is that sorafenib, a drug used in the treatment of recurrent or progressive malignant glioma,[183] sensitises the cells to small-molecule inhibitors of Bcl-2–like proteins in a STAT3-dependent manner.[184] Signal transducer and activator of transcription 3 is a transcription factor known to upregulate NNMT expression and that its activation is a major factor in tumour malignancy.[185–188]

Oral squamous cell carcinoma

Sartini et al were first to give a detailed account of NNMT upregulation in oral squamous cell carcinoma. Using RT-PCR and real-time quantitative PCR on cancerous and adjacent normal mucosa, they found that NNMT mRNA was more highly expressed. Nevertheless, in contrast to what may be seen in clear cell renal cell carcinoma, high expression was found in cancers from patients with the more favourable outcome. Little difference was found in NNMT expression when levels in cancerous tissue and normal mucosa from patients with a less favourable outcome were compared. Thus, NNMT expression was inversely correlated with the presence of lymph node metastases.[124] In accordance with these findings, cancers exhibiting high NNMT expression were more differentiated than ones with low expression.[125] Later, however, although high NNMT expression was linked to a more favourable outcome, cancer stem cells were found to have high expression,[134] and downregulation of NNMT by silencing mRNA reduced tumourigenicity both in in vitro and in vivo mouse assays.[189] Interestingly, NNMT expression also correlates inversely with prolonged progression-free and overall survival times in prostate cancer.[130] A further variation on the theme of NNMT expression may be seen in oral cancers likely resulting from chewing tobacco. Here, high expression was found in only approximately 50% of cases.[190]

Hepatocellular carcinoma

Nicotinamide *N*-methyltransferase is one of many genes that have been shown to be differentially regulated in hepatocellular carcinoma. Studying data amassed previously from large-scale studies contained in libraries, such as the Encyclopedia of Hepatocellular Carcinoma genes Online, and from reports by individual groups of authors[191,192] and from their own investigation, Hsu et al concluded that NNMT mRNA levels were downregulated in hepatocellular carcinoma.[193] Nevertheless, in a later study, Kim et al concluded from studying 120 cases of hepatocellular carcinoma at different stages that although NNMT expression may be lower in cancer than noncancerous tissue, relatively high NNMT mRNA expression was correlated with poor prognosis and a significantly shorter disease-free survival time ($P = .016$).[119] A further confounding factor in the interpretation of NNMT levels arises from the cause of the carcinoma. Infection with either hepatitis B virus or hepatitis C virus is a serious predisposing factor. Iizuka et al[194] showed that cancers resulting from infection with hepatitis B virus had lower levels of NNMT mRNA expression than patients whose cancers resulted from hepatitis C virus infection.

Conclusions

The dominant position of NNMT in NAM metabolism in humans was demonstrated by Mrochek et al as early as 1976. Initially, patients with schizophrenia were shown to have metabolic profiles little different from those of healthy subjects. Having established this, they fed 10 patients (1 healthy individual and 9 patients with schizophrenia) graded doses (100-3000 mg) of NAM and assayed 24-hour urine output of a range of metabolites (NAM, MeNAM, NAM-*N*1-oxide, *N*1-methyl-2-pyridone-5-carbamide, *N*1-methyl-4-pyridone-3-carbamide). At the highest dose of NAM after 24 hours, on average, approximately 44% of the ingested dose was recovered. Methylated metabolites formed 78% of recovered compounds, whereas nonmethylated compounds (NAM-*N*1-oxide 13% and NAM 9%) formed only 22% of compounds recovered in the urine.[195]

Research into the biochemistry and physiology of NNMT over the following decades has followed a number of recurring themes, from the early characterisation of NNMT's substrate specificities by Alston and Abeles[67] in 1988 working on the porcine enzyme, through Rini et al[37] a few years later exploring the human enzyme, to the latest report of van Haren et al,[196] who also characterised a similar range of substrates in great

detail. The idea that NNMT may be linked to the availability of methyl groups in vivo has developed from the early work of Kang-Lee et al,[197] who studied the effects of increasing doses of NAM in rats fed a casein diet, to the proposition of Ulanovskaya et al,[139] that NNMT created a metabolic sink for methyl groups in cancer. As a consequence of these recurring themes, the concept of NNMT has developed from that of being merely a phase 2 enzyme to one that is capable of shaping the destiny of the human from its earliest phase of life by modulating its epigenetic landscape.[50] Regarding the expression of NNMT in cancer, it is clear that in most of the cancers studied, NNMT expression is generally increased. Nevertheless, it is important to note that this increase does not occur in all cancers and neither does it occur in all individual patients even though the mean level of expression of a group of such patients is high. Furthermore, the effect of an increased expression in the malignant tissue is variable. In some cases, increased NNMT expression is associated with a more aggressive phenotype, and in other cases, this is not so. Whether a therapeutic use can be made of increased NNMT expression remains an underexplored area. One report has been published on the use of a pharmaceutical to interfere with expression – depsipeptide interaction with the HNF-1β response element in the NNMT gene promoter by Xu and Hershman[198] – but this was some 10 years ago. Another group in 2010 demonstrated the variable expression of NNMT in a human rhabdomyosarcoma cell line in response to different insulin-like growth factor I receptor antagonists.[199] This work increased the range of factors influencing NNMT expression, together with the effects associated with NNMT expression in cancer, which is illustrated in Figure 3. Since then, nothing on this topic has emerged. Whether this is because the topic has been explored and found to be unprofitable or has not been an area of research is unclear. In conclusion, it can be seen that much has emerged to reshape our concept of the function of NNMT, but numerous unanswered questions still exist.

Acknowledgements

DBR and co-authors wish to acknowledge the United Birmingham Hospitals Charity for its financial contribution to the publication of this work.

Author Contributions

All authors reviewed and agreed the final text. RHW contributed the section on Phase 2 metabolism. DJB contributed the section on NNMT structure. RBP contributed the section on enzyme kinetics. DBR was the main contributor.

REFERENCES

1. http://ensemblgenomes.org/search.
2. Rongvaux A, Andris F, Van Gool F, Leo O. Reconstructing eukaryotic NAD metabolism. *BioEssays*. 2003;25:683–690.
3. Lin H, Kwan AL, Dutcher SK. Synthesizing and salvaging NAD+: lessons learned from *Chlamydomonas reinhardtii*. *PLoS Genet*. 2010;6:e1001105.
4. De Figueiredo LF, Gossmann TI, Ziegler M, Schuster S. Pathway analysis of NAD+ metabolism. *Biochem J*. 2011;439:341–348.
5. Ebenhoh O, Heinrich R. Evolutionary optimization of metabolic pathways. Theoretical reconstruction of the stoichiometry of ATP and NADH producing systems. *B Math Biol*. 2001;63:21–55.
6. Gossmann TI, Ziegler M. Sequence divergence and diversity suggests ongoing functional diversification of vertebrate NAD metabolism. *DNA Repair*. 2014;23:39–48.
7. Gossmann TI, Ziegler M, Puntervoll P, de Figueiredo LF, Schuster S, Heiland I. NAD(+) biosynthesis and salvage—a phylogenetic perspective. *FEBS J*. 2012;279:3355–3363.
8. His W. Ueber das Stoffwechselproduct des Pyridins. *Arch Exp Pathol Pharmakol*. 1884;22:253–260.
9. Ellinger P, Coulson RA. The urinary elimination of nicotinamide methochloride by man. *Biochem J*. 1944;38:265–270.
10. Ellinger P, Abdel Kader MM. Nicotinamide metabolism in mammals. *Biochem J*. 1949;44:77–87.
11. Knox E, Grossman WI. A new metabolite of nicotinamide. *J Biol Chem*. 1946;166:391–392.
12. Holman WIM, Wiegand C. The chemical conversion of nicotinic acid and nicotinamide to derivatives of N-methyl-2-pyridone by methylation and oxidation. *Biochem J*. 1948;43:423–426.
13. Mann PJG, Quastel JH. Nicotinamide, cozymase and tissue metabolism. *Biochem J*. 1941;35:502–517.
14. Perlzweig WA, Bernheim MLC, Bernheim F. The methylation of nicotinamide by rat liver in vitro. *J Biol Chem*. 1943;150:401–406.
15. Cantoni GL. Methylation of nicotinamide with soluble enzyme system from rat liver. *J Biol Chem*. 1951;189:203–216.
16. Aksoy S, Brandriff BF, Ward A, Little PF, Weinshilboum RM. Human nicotinamide N-methyltransferase gene: molecular cloning, structural characterization and chromosomal localization. *Genomics*. 1995;29:555–561.
17. Aksoy S, Szumlanski CL, Weinshilboum RM. Human liver nicotinamide N-methyltransferase. cDNA cloning, expression, and biochemical characterization. *J Biol Chem*. 1994;269:14835–14840.
18. http://ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000166741;r=11:114257831-114313285;t=ENST00000535401.
19. http://diyhpl.us/~bryan/irc/protocol-online/protocol-cache/TFSEARCH.html.
20. Xu J, Capezzone M, Xu X, Hershman JM. Activation of nicotinamide N-methyltransferase gene promoter by hepatocyte nuclear factor-1β in human papillary thyroid cancer cells. *Mol Endocrinol*. 2005;19:527–539.
21. Tomida M, Ohtake H, Yokota T, Kobayashi Y, Kurosumi M. Stat3 up-regulates expression of nicotinamide N-methyltransferase in human cancer cells. *J Cancer Res Clin Oncol*. 2008;134:551–559.
22. Nabokikh A, Ilhan A, Bilban M, et al. Reduced TGF-beta1 expression and its target genes in human insulinomas. *Exp Clin Endocrinol Diabetes*. 2007;115:674–682.
23. Heemers HV, Schmidt LJ, Sun Z, et al. Identification of a clinically relevant androgen-dependent gene signature in prostate cancer. *Cancer Res*. 2011;71:1978–1988.
24. Katiyar S, Jiao X, Addya S, et al. Mammary gland selective excision of c-Jun identifies its role in mRNA splicing. *Cancer Res*. 2012;72:1023–1034.
25. Yan L, Otterness DM, Weinshilboum RM. Human nicotinamide N-methyltransferase pharmacogenetics: gene sequence analysis and promoter characterization. *Pharmacogenetics*. 1999;9:307–316.
26. Seshadri S, DeStefano AL, Au R, et al. Genetic correlates of brain aging on MRI and cognitive test measures: a genome-wide association and linkage analysis in the Framingham study. *BMC Med Genet*. 2007;8:S15.
27. de Jonge R, Tissing WJ, Hooijberg JH, et al. Polymorphisms in folate-related genes and risk of pediatric acute lymphoblastic leukemia. *Blood*. 2009;113:2284–2289.
28. Koppen IJ, Hermans FJ, Kaspers GJ. Folate related gene polymorphisms and susceptibility to develop childhood acute lymphoblastic leukaemia. *Br J Haematol*. 2010;148:3–14.
29. Roberg K, Ceder R, Farnebo L, Norberg-Spaak L, Grafström RC. Multiple genotypic aberrances associate to terminal differentiation-deficiency of an oral squamous cell carcinoma in serum-free culture. *Differentiation*. 2008;76:868–880.
30. van Driel LMJW, Smedts HPM, Helbing WA, et al. Eight-fold increased risk for congenital heart defects in children carrying the nicotinamide N-methyltransferase polymorphism and exposed to medicines and low nicotinamide. *Eur Heart J*. 2008;29:1424–1431.
31. Sazci A, Ozel MD, Ergul E, Onder ME. Association of nicotinamide-N-methyltransferase (NNMT) gene rs694539 variant with bipolar disorder. *Gene*. 2013;532:272–275.
32. Lim BH, Cho BI, Kim YN, Kim JW, Park ST, Lee CW. Overexpression of nicotinamide N-methyltransferase in gastric cancer tissues and its potential post-translational modification. *Exp Mol Med*. 2006;38:455–465.

33. Riederer M, Erwa W, Zimmermann R, Frank S, Zechner R. Adipose tissue as a source of nicotinamide N-methyltransferase and homocysteine. *Atherosclerosis*. 2009;204:412–417.
34. Parsons RB, Smith ML, Williams AC, Waring RH, Ramsden DB. Expression of nicotinamide N-methyltransferase (E.C. 2.1.1.1) in the Parkinsonian brain. *J Neuropathol Exp Neurol*. 2002;61:111–124.
35. Bromberg A, Lerer E, Udawela M, et al. Nicotinamide-N-methyltransferase (NNMT) in schizophrenia: genetic association and decreased frontal cortex mRNA levels. *Int J Neuropsychopharmacol*. 2012;15:727–737.
36. Thirumoorthy K, Gordon N, Walston S, et al. OM-19 Targeted metabolomic profiling identifies the substrates of nicotinamide-N-methyltransferase as a biomarker in glioblastoma. *Neuro Oncol*. 2012;14:vi91–vi100.
37. Rini J, Szumlanski C, Guerciolini R, Weinshilboum RM. Human liver nicotinamide N-methyltransferase: ion-pairing radiochemical assay, biochemical properties and individual variation. *Clin Chim Acta*. 1990;186:359–374.
38. Smith ML, Burnett D, Bennett P, et al. A direct correlation between nicotinamide N-methyltransferase activity and protein levels in human liver cytosol. *Biochim Biophys Acta*. 1998;1422:238–244.
39. Gomi T, Takata Y, Fujioka M. Rat liver S-adenosylhomocysteinase. Spectrophotometric study of coenzyme binding. *Biochim Biophys Acta*. 1989;994:172–179.
40. Martin JL, McMillan FM. SAM (dependent) I AM: the S-adenosylmethionine-dependent methyltransferase fold. *Curr Opin Struct Biol*. 2002;12:783–793.
41. Peng Y, Sartini D, Pozzi V, Wilk D, Emanuelli M, Yee VC. Structural basis of substrate recognition in human nicotinamide N-methyltransferase. *Biochemistry*. 2011;50:7800–7808.
42. Tomida M, Mikami I, Takeuchi S, Nishimura H, Akiyama H. Serum levels of nicotinamide N-methyltransferase in patients with lung cancer. *J Cancer Res Clin Oncol*. 2009;135:1223–1229.
43. Cuomo R, Dattilo M, Pumpo R, Capuano G, Boselli L, Budillon G. Nicotinamide methylation in patients with cirrhosis. *J Hepatol*. 1994;20:138–142.
44. Pumpo R, Sarnelli G, Spinella A, Budillon G, Cuomo R. The metabolism of nicotinamide in human liver cirrhosis: a study on N-methylnicotinamide and 2-pyridone-5-carboxamide production. *Am J Gastroenterol*. 2001;96:1183–1187.
45. Buttram S. *N-Methylation in Parkinson's Disease After Nicotinamide Administration* [MSc thesis]. Birmingham, UK: University of Birmingham; 1990.
46. Bailey SM, Udoh US, Young ME. Circadian regulation of metabolism. *J Endocrinol*. 2014;222:R75–R96.
47. Moseley RH, Morrissette J, Johnson TR. Transport of N1-methylnicotinamide by organic cation-proton exchange in rat liver membrane vesicles. *Am J Physiol*. 1990;259:G973–G982.
48. Redzic Z. Molecular biology of the blood-brain and the blood-cerebrospinal fluid barriers: similarities and differences. *Fluids Barriers CNS*. 2011;8:3.
49. Klaassen CD, Aleksunes LM. Xenobiotic, bile acid, and cholesterol transporters: function and regulation. *Pharmacol Rev*. 2010;62:1–96.
50. Lu H, Gunewardena S, Cui JY, Yoo B, Zhong XB, Klaassen CD. RNA-sequencing quantification of hepatic ontogeny and tissue distribution of mRNAs of phase II enzymes in mice. *Drug Metab Dispos*. 2013;41:844–857.
51. Sperber H, Mathieu J, Wang Y, et al. The metabolome regulates the epigenetic landscape during naïve-to-primed human embryonic stem cell transition. *Nat Cell Biol*. 2015;17:1523–1535.
52. Huffmann K. The developing, aging neocortex: how genetics and epigenetics influence early developmental patterning and age-related change. *Front Genet*. 2012;3:212.
53. Chouliaras L, van den Hove DL, Kenis G, et al. Caloric restriction attenuates age-related changes of DNA methyltransferase 3a in mouse hippocampus. *Brain Behav Immun*. 2011;25:616–623.
54. Chouliaras L, vanden Hove DL, Kenis G, et al. Prevention of age-related changes in hippocampal levels of 5-methylcytidine by caloric restriction. *Neurobiol Aging*. 2012;33:1672–1681.
55. Lin SJ, Defossez PA, Guarente L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science*. 2000;289:2126–2128.
56. Bitterman KJ, Anderson RM, Cohen HY, Latorre-Esteves M, Sinclair DA. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast Sir2 and human SIRT1. *J Biol Chem*. 2002;277:45099–45107.
57. Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Sinclair DA. Nicotinamide and *PNC1* govern lifespan extension by calorie restriction in *Saccharomyces cerevisiae*. *Nature*. 2003;423:181–185.
58. Imai S-I, Guarente L. NAD^+ and sirtuins in aging and disease. *Trends Cell Biol*. 2014;24:464–471.
59. Schmeisser K, Mansfeld J, Kuhlow D, et al. Role of sirtuins in lifespan regulation is linked to methylation of nicotinamide. *Nat Chem Biol*. 2013;9:693–700.
60. Estep PW III, Warner JB, Bulyk ML. Short-term calorie restriction in male mice feminizes gene expression and alters key regulators of conserved aging regulatory pathways. *PLoS ONE*. 2009;4:e5242.
61. Ghosh D, LeVault K, Barnett A, Brewer GJ. A reversible early oxidized redox state that precedes macromolecular ROS damage in aging nontransgenic and 3xTg-AD mouse neurons. *J Neurosci*. 2012;32:5821–5832.
62. O'Brown ZK, Van Nostrand EL, Higgins JP, Kim SK. The inflammatory transcription factors NFκB, STAT1 and STAT3 drive age-associated transcriptional changes in the human kidney. *Plos Genet*. 2015;12:e1005734.
63. Stanulović M, Chaykin S. Aldehyde oxidase: catalysis of the oxidation of N1-methylnicotinamide and pyridoxal. *Arch Biochem Biophys*. 1971;145:27–34.
64. Stanulovic M, Chaykin S. Metabolic origins of the pyridones on N-methylnicotinamide in man and rat. *Arch Biochem Biophys*. 1971;145:35–42.
65. Holman WIM, De Lange DJ. Methods for the determination of N-methyl-2-pyridone-5-carboxylamide and of N-methyl-2-pyridone-3-carboxylamide in human urine. *Biochem J*. 1949;45:559–562.
66. D'Souza J, Caldwell J, Smith RL. Species variations in the N-methylation and quaternization of [14C]pyridine. *Xenobiotica*. 1980;10:151–157.
67. Alston T, Abeles R. Substrate specificity of nicotinamide methyltransferase isolated from porcine liver. *Arch Biochem Biophys*. 1988;260:601–608.
68. Thomas MG, Sartini D, Emanuelli M, et al. Nicotinamide N-methyltransferase catalyses the *n*-methylation of the endogenous β-carboline norharman: evidence for a novel detoxification pathway. *Biochem J*. 2016;473:3253–3267.
69. Caldwell J, D'Souza J, Smith RL. Species variations in the N-methylation and quaternization of [^{14}C]-pyridine in vivo [proceedings]. *Br J Pharmacol*. 1980;68:119P.
70. Fukushima T, Kaetsu A, Lim H, Moriyama M. Possible role of 1-methylnicotinamide in the pathogenesis of Parkinson's disease. *Exp Toxicol Pathol*. 2002;53:469–473.
71. Nikiforov A, Dolle C, Niere M, et al. Pathways and subcellular compartmentation of NAD biosynthesis in human cells: from entry of extracellular precursors to mitochondrial NAD generation. *J Biol Chem*. 2011;286:21767–22178.
72. Yamamoto M, Hikosaka K, Mahmood A, et al. Nmnat3 is dispensable in mitochondrial NAD level maintenance in vivo. *PLoS ONE*. 2016;11:e0147037.
73. Scher MB, Vaquero A, Reinberg D. SirT3 is a nuclear NAD^+-dependent histone deacetylase that translocates to the mitochondria upon cellular stress. *Genes Dev*. 2007;21:920–928.
74. Wei W, Graeff R, Yue J. Roles and mechanisms of the CD38/cyclic adenosine diphosphate ribose/$Ca2^+$ signaling pathway. *World J Biol Chem*. 2014;5:58–67.
75. Bütepage M, Eckei L, Verheugd P, Lüscher B. Intracellular mono-ADP-ribosylation in signaling and disease. *Cells*. 2015;4:569–595.
76. MacPherson L, Tamblyn L, Rajendra S, Bralha F, McPherson JP, Matthews J. 2,3,7,8-Tetrachlorodibenzo-p-dioxin poly(ADP-ribose) polymerase (TiPARP, ARTD14) is a mono-ADP-ribosyltransferase and repressor of aryl hydrocarbon receptor transactivation. *Nucleic Acids Res*. 2013;41:1604–1621.
77. Gibson BA, Kraus WL. New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. *Nat Rev Mol Cell Biol*. 2012;13:411–424.
78. Sistigua A, Manica G, Obrist F, Vitale I. Trial watch—inhibiting PARP enzymes for anticancer therapy. *Mol Cell Oncol*. 2016;3:e1053594.
79. Gibson BA, Zhang Y, Jiang H, et al. Chemical genetic discovery of PARP targets reveals a role for PARP-1 in transcription elongation. *Science*. 2016;353:45–50.
80. Rajendran R, Garva R, Krstic-Demonacos M, Demonacos C. Sirtuins: molecular traffic lights in the crossroad of oxidative stress, chromatin remodeling, and transcription. *J Biomed Biotechnol*. 2011;2011:368276.
81. Chun SK, Go K, Yang M-J, Zendejas I, Behrns KE, Kim J-S. Autophagy in ischemic livers: a critical role of sirtuin 1/mitofusin 2 axis in autophagy induction. *Toxicol Res*. 2016;32:35–46.
82. Yuana Y, Cruzat VF, Newsholme P, Chenga J, Chena Y, Lua Y. Regulation of SIRT1 in aging: roles in mitochondrial function and biogenesis. *Mech Ageing Dev*. 2016;155:10–21.
83. Ido Y. Diabetic complications within the context of aging: nicotinamide adenine dinucleotide redox, insulin C-peptide, sirtuin 1-liver kinase B1-adenosine monophosphate-activated protein kinase positive feedback and forkhead box O3. *J Diabetes Investig*. 2016;7:448–458.
84. Kong X, Wang R, Xue Y, et al. Sirtuin 3, a new target of PGC-1α, plays an important role in the suppression of ROS and mitochondrial biogenesis. *PLoS ONE*. 2010;5:e11707.
85. George J, Ahmad N. Mitochondrial sirtuins in cancer: emerging roles and therapeutic potential. *Cancer Res*. 2016;76:2500–2506.
86. Bugger H, Witt CN, Bode C. Mitochondrial sirtuins in the heart. *Heart Fail Rev*. 2016;21:519–528.

87. Kugel S, Mostoslavsky R. Chromatin and beyond: the multitasking roles for SIRT6. *Trends Biochem Sci*. 2014;39:72–81.
88. Abdelraheim SR, Spiller DG, Mclennan AG. Mammalian NADH diphosphatases of the Nudix family: cloning and characterization of the human peroxisomal NUDT12 protein. *Biochem J*. 2003;374:329–335.
89. Opitz CA, Heiland I. Metabolic pathways analysis 2015: dynamics of NAD-metabolism: everything but constant. *Biochem Soc Trans*. 2015;43:1127–1132.
90. Verdin E. NAD in aging, metabolism and neurodegeneration. *Science*. 2015;350:1208–1213.
91. Nikiforov A, Kulikova V, Ziegler M. The human NAD metabolome: functions, metabolism and compartmentalization. *Crit Rev Biochem Mol Biol*. 2015;50:284–297.
92. *The National Diet and Nutrition Survey: People Aged 65 Years and Over*; vol 1. London, England: The Stationary Office; 1998:194–223.
93. Mayneris-Perxachs J, Lima AAM, Guerrant RL, et al. Urinary N-methylnicotinamide and β-aminoisobutyric acid predict catch-up growth in undernourished Brazilian children. *Sci Rep*. 2016;6:9780.
94. Chalkiadaki A, Guarente L. Metabolic signals regulate SIRT1 expression. *EMBO Rep*. 2011;12:985–986.
95. Nogueiras R, Habegger KM, Chaudhary N, et al. Sirtuin 1 and sirtuin 3: physiological modulators of metabolism. *Physiol Rev*. 2012;92:1479–1514.
96. Kraus D, Yang Q, Kong D, et al. Nicotinamide N-methyltransferase knockdown protects against diet-induced obesity. *Nature*. 2014;508:258–262.
97. Kannt A, Pfenninger A, Teichert L, et al. Association of nicotinamide-N-methyltransferase mRNA expression in human adipose tissue and the plasma concentration of its product, 1-methylnicotinamide, with insulin resistance. *Diabetologia*. 2015;58:799–808.
98. Liu M, Li L, Chu J, et al. Serum N(1)-methylnicotinamide is associated with obesity and diabetes in Chinese. *J Clin Endocrinol Metab*. 2015;100:3112–3117.
99. Liu M, Li L, Chu J, et al. Os 33-03 serum N1-methylnicotinamide is associated with obesity and diabetes in Chinese. *J Hypertens*. 2016;34:e393–e394.
100. Hong S, Moreno-Navarrete JM, Wei X, et al. Nicotinamide N-methyltransferase regulates hepatic nutrient metabolism through Sirt1 protein stabilization. *Nat Med*. 2015;21:887–894.
101. Rodgers JT, Puigserver P. Fasting-dependent glucose and lipid metabolic response through hepatic sirtuin 1. *Proc Natl Acad Sci U S A*. 2007;104:12861–12866.
102. Liu Y, Dentin R, Chen D, et al. A fasting inducible switch modulates gluconeogenesis via activator/coactivator exchange. *Nature*. 2008;456:269–273.
103. Erion DM, Yonemitsu S, Nie Y, et al. SirT1 knockdown in liver decreases basal hepatic glucose production and increases hepatic insulin responsiveness in diabetic rats. *Proc Natl Acad Sci U S A*. 2009;106:11288–11293.
104. Ponugoti B, Kim D-H, Xiao Z, et al. SIRT1 deacetylates and inhibits SREBP-1C activity in regulation of hepatic lipid metabolism. *J Biol Chem*. 2010;285:33959–33970.
105. Walker AK, Yang F, Jiang K, et al. Conserved role of SIRT1 orthologs in fasting-dependent inhibition of the lipid/cholesterol regulator SREBP. *Genes Dev*. 2010;24:1403–1417.
106. Liu L, Nam M, Fan W, et al. Nutrient sensing by the mitochondrial transcription machinery dictates oxidative phosphorylation. *J Clin Invest*. 2014;124:768–784.
107. Parsons RB, Smith SH, Waring RH, Williams AC, Ramsden DB. High expression of nicotinamide N-methyltransferase in patients with idiopathic Parkinson's disease. *Neurosci Lett*. 2003;342:13–16.
108. Kuchmerovska T, Shymanskyy I, Chlopickib S, Klimenko A. 1-methylnicotinamide (MNA) in prevention of diabetes-associated brain disorders. *Neurochem Int*. 2010;56:221–228.
109. Trammell SA, Brenner C. NNMT: a bad actor in fat makes good in liver. *Cell Metab*. 2015;22:200–201.
110. Cantó1 C, Menzies K, Auwerx J. NAD^+ metabolism and the control of energy homeostasis—a balancing act between mitochondria and the nucleus. *Cell Metab*. 2015;22:31–53.
111. Zhou S-S, Li D, Chen N-N, Zhou Y. Vitamin paradox in obesity: deficiency or excess? *World J Diabetes*. 2015;6:158–1167.
112. Xu J, Moatamed F, Caldwell JS, et al. Enhanced expression of nicotinamide N-methyltransferase in human papillary thyroid carcinoma cells. *J Clin Endocrinol Metab*. 2003;88:4990–4996.
113. Riester M, Taylor JM, Feifer A, et al. Combination of a novel gene expression signature with a clinical nomogram improves the prediction of survival in high-risk bladder cancer. *Clin Cancer Res*. 2012;18:1323–1333.
114. Sartini D, Muzzonigro G, Milanese G, et al. Upregulation of tissue and urinary nicotinamide N-methyltransferase in bladder cancer: potential for the development of a urine-based diagnostic test. *Cell Biochem Biophys*. 2013;65:473–483.
115. Kuo W-H, Chang Y-Y, Lai L-C, et al. Molecular characteristics and metastasis predictor genes of triple-negative breast cancer: a clinical study of triple-negative breast carcinomas. *PLoS ONE*. 2012;7:e45831.
116. Roessler M, Rollinger W, Palme S, et al. Identification of nicotinamide N-methyltransferase as a novel serum tumor marker for colorectal cancer. *Clin Cancer Res*. 2005;11:6550–6557.
117. Chen C, Wang X, Huang X, et al. Nicotinamide N-methyltransferase: a potential biomarker for worse prognosis in gastric carcinoma. *Am J Cancer Res*. 2016;6:649–663.
118. Li A, Walling J, Ahn S, et al. Unsupervised analysis of transcriptomic profiles reveals six glioma subtypes. *Cancer Res*. 2009;69:2091–2099.
119. Kim J, Hong SJ, Lim EK, et al. Expression of nicotinamide N-methyltransferase in hepatocellular carcinoma is associated with poor prognosis. *J Exp Clin Cancer Res*. 2009;28:20.
120. Sartini D, Seta R, Pozzi V, et al. Role of nicotinamide N-methyltransferase in non-small cell lung cancer: in vitro effect of shRNA-mediated gene silencing on tumourigenicity. *Biol Chem*. 2015;396:225–234.
121. Piccaluga PP, Agostinelli C, Califano A, et al. Gene expression analysis of peripheral T cell lymphoma, unspecified, reveals distinct profiles and new potential therapeutic targets. *J Clin Invest*. 2007;117:823–834.
122. Piccaluga PP, Agostinelli C, Califano A, et al. Gene expression analysis of angioimmunoblastic lymphoma indicates derivation from T follicular helper cells and vascular endothelial growth factor deregulation. *Cancer Res*. 2007;67:10703–10710.
123. Win KT, Lee SW, Huang HY, et al. Nicotinamide N-methyltransferase overexpression is associated with Akt phosphorylation and indicates worse prognosis in patients with nasopharyngeal carcinoma. *Tumour Biol*. 2013;34:3923–3931.
124. Sartini D, Santarelli A, Rossi V, et al. Nicotinamide N-methyltransferase upregulation inversely correlates with lymph node metastasis in oral squamous cell carcinoma. *Mol Med*. 2007;13:415–421.
125. Emanuelli M, Santarelli A, Sartini D, et al. Nicotinamide N-methyltransferase upregulation correlates with tumour differentiation in oral squamous cell carcinoma. *Histol Histopathol*. 2010;25:15–20.
126. Jiang Q, Yu Y-C, Ding X-J, Luo Y, Ruan H. Bioinformatics analysis reveals significant genes and pathways to target for oral squamous cell carcinoma. *Asian Pac J Cancer Prev*. 2014;15:2274–2278.
127. Bignotti E, Tassi RA, Calza S, et al. Gene expression profile of ovarian serous papillary carcinomas: identification of metastasis-associated genes. *Am J Obstet Gynecol*. 2007;196:245.e1-11.
128. Rogers CD, Fukushima N, Sato N, et al. Differentiating pancreatic lesions by microarray and QPCR analysis of pancreatic juice RNAs. *Cancer Biol Ther*. 2006;5:1383–1389.
129. Xu Y, Liu P, Zheng DH, et al. Expression profile and prognostic value of NNMT in patients with pancreatic cancer. *Oncotarget*. 2016;7:19976–19981.
130. Zhou W, Gui M, Zhu M, et al. Nicotinamide N-methyltransferase is overexpressed in prostate cancer and correlates with prolonged progression-free and overall survival times. *Oncol Lett*. 2014;8:1175-1180.
131. Hoshino J, Kijhne U, Kroger H. Enhancement of DNA synthesis and cell proliferation by L-methylnicotinamide in rat liver cells in culture: implication for its *in vivo* role. *Biochem Biophys Res Commun*. 1982;105:1446–1452.
132. Hoshino J, Kijhne U, Kroger H. Methylation of nicotinamide in rat liver cytosol and its correlation with hepatocellular proliferation. *Biochim Biophys Acta*. 1982;719:518–526.
133. Hoshino J, Schluter U, Kruger H. Nicotinamide methylation and its relation to NAD synthesis in rat liver tissue culture. Biochemical basis for the physiological activities of 1-methylnicotinamide. *Biochim Biophys Acta*. 1984;801:250–258.
134. Pozzia V, Sartini D, Rocchetti R, et al. Identification and characterization of cancer stem cells from head and neck squamous cell carcinoma cell lines. *Cell Physiol Biochem*. 2015;36:784–790.
135. Jung JE, Lee HG, Cho IH, et al. STAT3 is a potential modulator of HIF-1-mediated VEGF expression in human renal carcinoma cells. *FASEB J*. 2005;19:1296–1298.
136. Carruthers C, Suntzeff V. Nicotinamide content of some normal and malignant tissues; the apparent absence of niacin in epidermis. *Cancer Res*. 1952;12:879–885.
137. Kerr WK, Barkin M, Levers PE, Woo SK, Menczyk Z. The effect of cigarette smoking on bladder carcinogens in man. *Can Med Assoc J*. 1965;93:1–7.
138. Clark BR, Murai JT, Pomeranz A, Mills PA, Halpern RM. Altered distribution and excretion of N-1-methylnicotinamide in rats with Walker 256 carcinosarcoma. *Cancer Res*. 1975;35:1727–1733.
139. Ulanovskaya OA, Zuhl AM, Cravatt BF. NNMT promotes epigenetic remodeling in cancer by creating a metabolic methylation sink. *Nat Chem Biol*. 2013;9:300–306.
140. Shlomi T, Rabinowitz JD. Cancer mistunes methylation. *Nat Chem Biol*. 2013;9:293–294.
141. Parsons RB, Aravindan S, Kadampeswaran A, et al. The expression of nicotinamide N-methyltransferase increases ATP synthesis and protects SH-SY5Y neuroblastoma cells against the toxicity of Complex I inhibitors. *Biochem J*. 2011;436:145–155.

142. Liu KY, Mistry RJ, Aguirre CA, et al. Nicotinamide N-methyltransferase increases Complex I activity in SH-SY5Y cells via sirtuin 3. *Biochem Biophys Res Commun*. 2015;467:491–496.
143. Milani ZH, Ramsden DB, Parsons RB. Neuroprotective effects of nicotinamide N-methyltransferase and its metabolite 1-methylnicotinamide. *J Biochem Mol Toxicol*. 2013;27:451–456.
144. Tang SW, Yang TC, Lin WC, et al. Nicotinamide N-methyltransferase induces cellular invasion through activating matrix metalloproteinase-2 expression in clear cell renal cell carcinoma cells. *Carcinogenesis*. 2011;32:138–145.
145. Wu Y, Siadaty MS, Berens ME, Hampton GM, Theodorescu D. Overlapping gene expression profiles of cell migration and tumor invasion in human bladder cancer identify metallothionein 1E and nicotinamide N-methyltransferase as novel regulators of cell migration. *Oncogene*. 2008;27:6679–6689.
146. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. *J Gen Physiol*. 1927;8:519–530.
147. Capello M, Ferri-Borgogno S, Riganti C, et al. Targeting the Warburg effect in cancer cells through ENO1 knockdown rescues oxidative phosphorylation and induces growth arrest. *Oncotarget*. 2015;7:5598–5612.
148. Yu T, Wang YT, Chen P, et al. Effects of nicotinamide N -methyltransferase on PANC-1 cells proliferation, metastatic potential and survival under metabolic stress. *Cell Physiol Biochem*. 2015;35:710–721.
149. Yamada K, Miyazaki T, Hara N, Tsuchiya M. Interferon-gamma elevates nicotinamide N-methyltransferase activity and nicotinamide level in human glioma cells. *J Nutr Sci Vitaminol*. 2010;56:83–86.
150. Xie X, Yu H, Wang Y, et al. Nicotinamide N-methyltransferase enhances the capacity of tumorigenesis associated with the promotion of cell cycle progression in human colorectal cancer cells. *Arch Biochem Biophys*. 2014; 564:52–66.
151. Zhang J, Wang Y, Li G, Yu H, Xie X. Down-Regulation of nicotinamide N-methyltransferase induces apoptosis in human breast cancer cells via the mitochondria-mediated pathway. *PLoS ONE*. 2014;9:e89202.
152. Klein T, Bischoff R. Physiology and pathophysiology of matrix metalloproteases. *Amino Acids*. 2011;41:271–290.
153. Pozzi V, Mazzotta M, Muzio L, et al. Inhibiting proliferation in KB cancer cells by RNA interference-mediated knockdown of nicotinamide N-methyltransferase expression. *Int J Immunopathol Pharmacol*. 2011;24:69–77.
154. Blazejczyk A, Switalska M, Chlopicki S, et al. 1-Methylnicotinamide and its structural analog 1,4-dimethylpyridine for the prevention of cancer metastasis. *J Exp Clin Cancer Res*. 2016;35:110.
155. Feldmann G, Habe N, Dhara S, et al. Hedgehog inhibition prolongs survival in a genetically engineered mouse model of pancreatic cancer. *Gut*. 2008;57:1420–1430.
156. Bi H-C, Pan Y-Z, Qiu J-X, et al. N-methylnicotinamide and nicotinamide N-methyltransferase are associated with microRNA-1291-altered pancreatic carcinoma cell metabolome and suppressed tumorigenesis. *Carcinogenesis*. 2014;35:2264–2272.
157. D'Andrea FP, Safwat A, Kassem M, Gautier L, Overgaard J, Horsman MR. Cancer stem cell overexpression of nicotinamide N-methyltransferase enhances cellular radiation resistance. *Radiother Oncol*. 2011;99:373–378.
158. D'Andrea FP. Intrinsic radiation resistance of mesenchymal cancer stem cells and implications for treatment response in a murine sarcoma model. *Dan Med J*. 2012;59:B4388.
159. Kassem HS, Sangar V, Cowan R, Clarke N, Margison GP. A potential role of heat shock proteins and nicotinamide N-methyl transferase in predicting response to radiation in bladder cancer. *Int J Cancer*. 2002;101:454–460.
160. Hsu YC, Chen HY, Yuan S, et al. Genome-wide analysis of three-way interplay among gene expression, cancer cell invasion and anti-cancer compound sensitivity. *BMC Med*. 2013;11:106.
161. Kwon Y, Song J, Lee H, et al. Design, synthesis, and biological activity of sulfonamide analogues of antofine and cryptopleurine as potent and orally active antitumor agents. *J Med Chem*. 2015;58:7749–7762.
162. Kerr WK, Barkin M, Levers PE, Woo SK, Menczyk Z. The effect of cigarette smoking on bladder carcinogens in man. *Can Med Assoc J*. 1965;93:1–7.
163. Basu TK, Raven RW, Bates C, Williams DC. Excretion of 5-hydroxyindole acetic acid and N1-methylnicotinamide in advanced cancer patients. *Eur J Cancer*. 1973;9:527–528.
164. Bünger S, Haug U, Kelly M, et al. A novel multiplex-protein array for serum diagnostics of colon cancer: a case-control study. *BMC Cancer*. 2012;12:393.
165. Ujiie H, Tomida MO, Akiyama H, et al. Serum hepatocyte growth factor and interleukin-6 are effective prognostic markers for non-small cell lung cancer. *Anticancer Res*. 2012;32:3251–3258.
166. Su Kim D, Choi YD, Moon M, et al. Composite three-marker assay for early detection of kidney cancer. *Cancer Epidemiol Biomarkers Prev*. 2013;22:390–398.
167. Sartini D, Pozzi V, Renzi E, et al. Analysis of tissue and salivary nicotinamide N-methyltransferase in oral squamous cell carcinoma: basis for the development of a noninvasive diagnostic test for early-stage disease. *Biol Chem*. 2012;393:505–511.
168. Teng PN, Hood BL, Sun M, Dhir R, Conrads TP. Differential proteomic analysis of renal cell carcinoma tissue interstitial fluid. *J Proteome Res*. 2011;10:1333–1342.
169. Yao M, Tabuchi H, Nagashima Y, et al. Gene expression analysis of renal carcinoma: adipose differentiation-related protein as a potential diagnostic and prognostic biomarker for clear-cell renal carcinoma. *J Pathol*. 2005;205:377–387.
170. Jones J, Otu H, Spentzos D, et al. Gene signatures of progression and metastasis in renal cell cancer. *Clin Cancer Res*. 2005;11:5730–5739.
171. Zhang J, Xie XY, Yang SW, Wang J, He C. Nicotinamide N-methyltransferase protein expression in renal cell cancer. *J Zhejiang Univ Sci B*. 2010;11:136–143.
172. Teng PN, Hood BL, Sun M, Dhir R, Conrads TP. Differential proteomic analysis of renal cell carcinoma tissue interstitial fluid. *J Proteome Res*. 2011;10:1333–1342.
173. Girgis AH, Iakovlev VV, Beheshti B, et al. Multilevel whole-genome analysis reveals candidate biomarkers in clear cell renal cell carcinoma. *Cancer Res*. 2012;72:5273–5284.
174. Atrih A, Mudaliar MAV, Zakikhani P, et al. Quantitative proteomics in resected renal cancer tissue for biomarker discovery and profiling. *Br J Cancer*. 2014;110:1622–1633.
175. Zaravinos A, Pieri M, Mourmouras N, et al. Altered metabolic pathways in clear cell renal cell carcinoma: a meta-analysis and validation study focused on the deregulated genes and their associated networks. *Oncoscience*. 2014;1:117–131.
176. Eikrem O, Beisland C, Hjelle K, et al. Transcriptome sequencing (RNAseq) enables utilization of formalin-fixed, paraffin-embedded biopsies with clear cell renal cell carcinoma for exploration of disease biology and biomarker development. *PLoS ONE*. 2016;11:e0149743.
177. Moffitt RA, Yin-Goen Q, Stokes TH, et al. caCORRECT2: improving the accuracy and reliability of microarray data in the presence of artifacts. *BMC Bioinformatics*. 2011;12:383.
178. Wozniak MB, Le Calvez-Kelm F, Abedi-Ardekani B, et al. Integrative genome-wide gene expression profiling of clear cell renal cell carcinoma in Czech Republic and in the United States. *PLoS ONE*. 2013;8:e57886.
179. Cifola I, Spinelli R, Beltrame L, et al. Genome-wide screening of copy number alterations and LOH events in renal cell carcinomas and integration with gene expression profile. *Mol Cancer*. 2008;7:6.
180. Arai E, Sakamoto H, Ichikawa H, et al. Multilayer-omics analysis of renal cell carcinoma, including the whole exome, methylome and transcriptome. *Int J Cancer*. 2014;135:1330–1342.
181. Neely BA, Wilkins CE, Marlow LA, et al. Proteotranscriptomic analysis reveals stage specific changes in the molecular landscape of clear-cell renal cell carcinoma. *PLoS ONE*. 2016;11:e0154074.
182. Fisher KE, Yin-Goen Q, Alexis D, et al. Gene expression profiling of clear cell papillary renal cell carcinoma: comparison with clear cell renal cell carcinoma and papillary renal cell carcinoma. *Mod Pathol*. 2014;27:222–230.
183. Nabors LB, Supko JG, Rosenfeld M, et al. Phase I trial of sorafenib in patients with recurrent or progressive malignant glioma. *Neuro Oncol*. 2011;13:1324–1330.
184. Kiprianova I, Remy J, Milosch N, et al. Sorafenib sensitizes glioma cells to the BH3 mimetic ABT-737 by targeting MCL1 in a STAT3-dependent manner. *Neoplasia*. 2015;17:564–573.
185. Weissenberger J, Loeffler S, Kappeler A, et al. IL-6 is required for glioma development in a mouse model. *Oncogene*. 2004;23:3308–3316.
186. Gray GK, McFarland BC, Nozell SE, Benveniste EN. NF-κB and STAT3 in glioblastoma: therapeutic targets coming of age. *Expert Rev Neurother*. 2014;14:1293–1306.
187. Birner P, Toumangelova-Uzeir K, Natchev S, Guentchev M. STAT3 tyrosine phosphorylation influences survival in glioblastoma. *J Neurooncol*. 2010;100:339–343.
188. Priester M, Copanaki E, Vafaizadeh V, et al. STAT3 silencing inhibits glioma single cell infiltration and tumor growth. *Neuro Oncol*. 2013;15:840–852.
189. Pozzi V, Sartini D, Morganti S, et al. RNA-mediated gene silencing of nicotinamide N-methyltransferase is associated with decreased tumorigenicity in human oral carcinoma cells. *PLoS ONE*. 2013;8:e71272.
190. Chakrabarti S, Multani S, Dabholkar J, Saranath D. Whole genome expression profiling in chewing-tobacco-associated oral cancers: a pilot study. *Med Oncol*. 2015;32:60.
191. Kim M-Y, Park E, Park J-H, et al. Expression profile of nine novel genes differentially expressed in hepatitis B virus-associated hepatocellular carcinomas. *Oncogene*. 2001;20:4568–4575.

192. Okabe H, Satoh S, Kato T, et al. Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: identification of genes involved in viral carcinogenesis and tumor progression. *Cancer Res*. 2001;61:2129–2137.
193. Hsu C-N, Lai J-M, Liu C-H, et al. Detection of the inferred interaction network in hepatocellular carcinoma from EHCO (encyclopedia of hepatocellular carcinoma genes online). *BMC Bioinformatics*. 2007;8:66.
194. Iizuka N, Oka M, Yamada-Okabe H, et al. Comparison of gene expression profiles between hepatitis B virus and hepatitis C virus-infected hepatocellular carcinoma by oligonucleotide microarray data on the basis of a supervised learning method. *Cancer Res*. 2002;62:3939–3944.
195. Mrochek JE, Jolley RL, Young DS, Turner WJ. Metabolic response of humans to ingestion of nicotinic acid and nicotinamide. *Clin Chem*. 1976;22:1821–1827.
196. van Haren MJ, Toraño JS, Sartini D, Emanuelli M, Parsons RB, Martin NI. A rapid and efficient assay for the characterization of substrates and inhibitors of nicotinamide N-methyltransferase. *Biochemistry*. 2016;55:5307–5315.
197. Kang-Lee YA, McKee RW, Wright SM, Swendseid ME, Jenden DJ, Jope RS. Metabolic effects of nicotinamide administration in rats. *J Nutr*. 1983;113:215–221.
198. Xu J, Hershman JM. Histone deacetylase inhibitor depsipeptide represses nicotinamide N-methyltransferase and hepatocyte nuclear factor-1β gene expression in human papillary thyroid cancer cells. *Thyroid*. 2006;16:151–160.
199. Huang F, Hurlburt W, Greer A, et al. Differential mechanisms of acquired resistance to insulin-like growth factor-I receptor antibody therapy or to a small-molecule inhibitor, BMS-754807, in a human rhabdomyosarcoma model. *Cancer Res*. 2010;70:7221–7231.